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THE SALIVARY FACTOR AND ITS RELATION TO DENTAL CARIES AND IMMUNITY IN DEMENTIA PRAECOX AND EPILEPSY

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Received for publication January 4, 1916

In a previous paper (1) it has been shown that a definite relationship exists between the neutralizing power of the normal resting saliva and that of the activated saliva. This relation, called the salivary factor, has been found to be indicative of the incidence of susceptibility to dental caries and of immunity therefrom, but is independent of oral cleanliness or the lack of it. The factor, while *not* an infallible test, has suggested a theory of one of the causes underlying the conditions of acquired and of absolute immunity.

In bacteriological work we find that by altering the acidity or the alkalinity of a culture medium, the growth of the organism may be inhibited. The application of this principle to oral conditions is obvious. Assuming the correctness of the salivary factor it will be found that in the condition of dental caries, the relationship of the neutralizing power of the activated saliva to that of the normal resting saliva varies only between relatively narrow limits. With immunity, the conditions are exactly the reverse and the relationship varies within relatively wide limits. The lactic acid-forming organisms, although capable of growth in a relatively high alkaline medium, flourish *more readily* in one of lesser alkalinity, such as may be furnished by the saliva found associated with caries. With the increase of growth of these bacteria there is a corresponding increase in the amount of lactic acid formed and the consequent solution of the calcium salts of the tooth structure.

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In the further consideration of the neutralizing power the question arose as to whether the salivary factor is as constant in certain types of nervous disorders as it is in the normal individual.

A series of analyses were made on exactly the same lines as formally the cases being: first dementia praecox; second, epilepsy.

Contrary to the expectations of several members of the medical staff of the different hospitals as well as to the writer, it was found that coöperation, in securing samples from dementia praecox patients, was better in the more acute types. Those, on the other hand, who were of a higher mentality failed in nearly every instance to give the necessary voluntary aid. The majority of cases reported are women for it was necessary to eliminate the undetermined influence of the tobacco stimulus, which has curtailed the work, to a certain extent, among the men. Only typical cases of caries and of immunity were chosen. The reports are in terms of cubic centimeters of two-hundredth normal solutions and are based on 10 cc. of saliva as sample.

In these tests, as well as in those previously reported, the *exact* H^+ ion concentration in the saliva is not the determination which is sought. What is measured is a quite different quantity and one which is probably much less subject to adventitious variation, namely, the *power of the saliva to maintain its H^+ ion concentration near to that of neutrality*. We determine, by means of arbitrarily chosen indicators, corresponding to arbitrarily chosen H^+ ion concentrations, on either side of absolute neutrality, the amount of reagent (acid or alkali) required to change the H^+ ion concentration of the saliva from the one arbitrarily selected value to the other. The greater this amount the greater is the power of the saliva to maintain an H^+ ion concentration which lies between these limits in the neighborhood of absolute neutrality. The quantity thus determined is, therefore, correctly to be regarded as a measure of the "*neutralizing power*" of the saliva. The salivary factor is the ratio of the neutralizing power of the normal resting saliva to that of the saliva activated by chewing paraffin, expressed in percentage.

In table 1 is given the results of analyses from those patients whose teeth were at the time free from caries and who were denoted as possessing present immunity. It will be observed that the salivary factor is below 80 per cent and therefore, indicative throughout of this condition. A peculiar fact, which has been brought out in this series of tests, is, that in over 42 per cent of the cases, the activated saliva was *alkaline*, instead of acid, to phenolphthalein. In the reporting of these

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TABLE 1

Dementia praecox

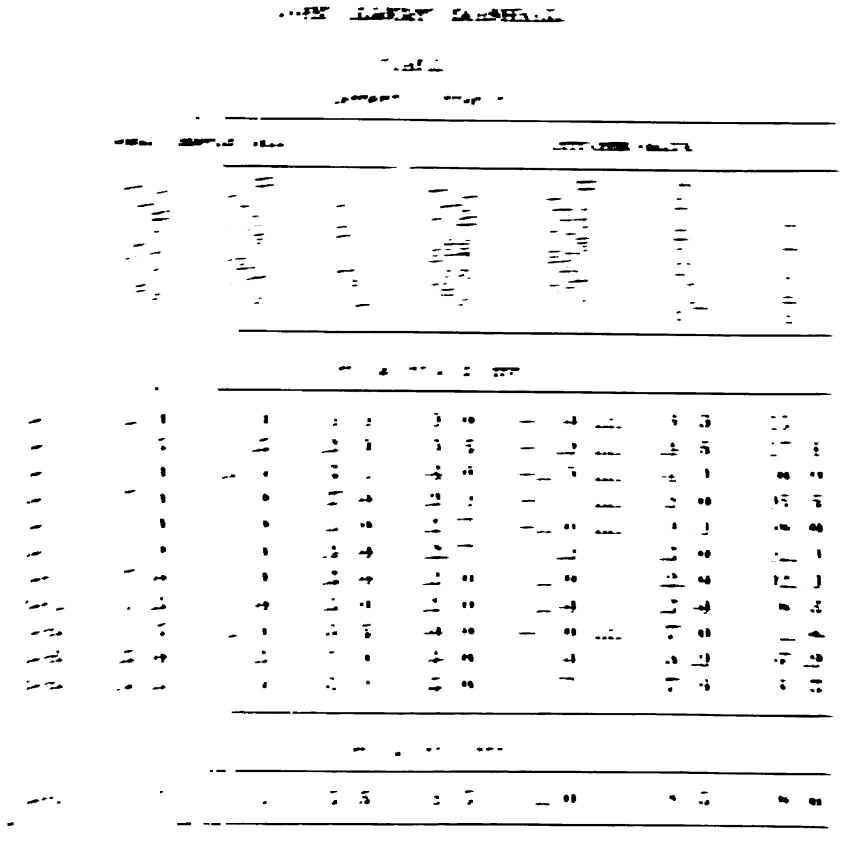
No. of patient	NORMAL RESTING SALIVA: Neutrality to P-nitro-phenol = cc. N/200 HCl	NORMAL RESTING SALIVA: Neutrality to phenolphthalein = cc. N/200 NaOH	NORMAL RESTING SALIVA: Neutralising power	ACTIVATED SALIVA: Neutrality to P-nitro-phenol = cc. N/200 HCl	ACTIVATED SALIVA: Neutrality to phenolphthalein = cc. N/200 NaOH	ACTIVATED SALIVA: Neutralising power	Salivary factor
Present immunity without care							
B- 5	12.60	4.80	17.40	37.05	−2.00 alk.	35.05	49.64
B-13	36.80	14.40	51.20	No coöperation			
B-14	8.00	31.60	39.60	No coöperation			
B-21	13.70	4.60	18.30	31.40	−3.50 alk.	27.90	65.59
Present immunity with care							
B- 3	12.10	9.30	22.40	37.30	4.00	41.30	54.24
B-22	9.50	5.50	15.00	26.90	0.95	27.85	53.86
B-23	18.55	1.90	20.45	32.00	−4.75 alk.	27.25	75.10
B-25	17.55	5.30	22.85	48.20	−3.60 alk.	44.60	51.23

analyses, the alkalinity, as indicated by a minus sign, has been deducted, for the determination of the neutralizing power, from the alkalinity as found with para-nitro-phenol. This alkalinity to phenolphthalein appears to be irrespective of oral conditions as it was found in both caries and immunity.

In table 2, the tabulations are compiled from analyses of the saliva found associated with dental caries. According to the reports previously made upon the normal individual, the salivary factor should be above 80 per cent. These findings are again confirmed in the following figures. It will likewise again be noted that there is a marked lack of uniformity in the acidity determinations, but in spite of this irregularity, however, the salivary factor remains remarkably constant.

In the case of a cretin, patient B-26, two separate analyses are given. The variation of a little over 5 per cent may be considered as within the limits of the experimental error; for the neutralizing power of both normal resting saliva and activated saliva checks to within 2 per cent. This patient was a man sixty years old and an inmate since 1881. The bony development in this particular case is noteworthy. The zygoma, mandible, and mastoid process are sensitive to pressure. About an

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TABLE 3

Epilepsy

No. of patient	Date of last recorded convulsion	Severity of convulsion	Time at which sample was secured	NORMAL RESTING SALIVA: Neutrality to P-nitro phenol = cc. N/200 HCl	NORMAL RESTING SALIVA: Neutrality to phenolphthalein = cc. N/200 NaOH	NORMAL RESTING SALIVA: Neutralising power	ACTIVATED SALIVA: Neutrality to P-nitro phenol = cc. N/200 HCl	ACTIVATED SALIVA: Neutrality to phenolphthalein = cc. N/200 NaOH	ACTIVATED SALIVA: Neutralising power	Salivary factor
Immunity without care										
										per cent
C-30	July 9	severe	July 12	22.80	2.65	25.45	36.25	3.30	39.55	64.35
C-31	July 6	severe	July 12	22.55	1.40	23.95	44.25	1.45	45.70	52.41
C-36	July 6	severe	July 13	14.85	5.95	20.80	38.05	−2.60 alk.	35.45	58.67
C-39	July 6	severe	July 13	8.90	4.45	13.45	24.70	1.95	26.65	50.09
C-44	June 6	light	July 13	23.60	2.20	25.80	44.80	−5.55 alk.	39.25	65.73
C-47	May 15	light	July 14	27.25	1.10	28.35	53.25	−4.50 alk.	48.75	58.15
C-48	July 7	light	July 14	16.05	6.40	22.45	38.05	−1.40 alk.	36.65	61.24
C-49	July 5	light	July 14	17.90	5.90	23.80	52.25	1.75	54.00	44.07
C-52	July 12	light	July 14	23.65	2.00	25.65	25.30	5.30	30.60	83.81
C-54	July 7	severe	July 14	20.25	3.30	23.55	38.90	−3.80 alk.	35.10	67.10

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TABLE 4

Epilepsy

No. of patient	Date of last recorded convulsion	Severity of convulsion	Time at which sample was secured	NORMAL RESTING SALIVA: Neutrality to P-nitro phenol = cc. N/200 HCl	NORMAL RESTING SALIVA: Neutrality to phenolphthalein = cc. N/200 Na_2OH	NORMAL RESTING SALIVA: Neutralising power	ACTIVATED SALIVA: Neutrality to P-nitro phenol = cc. N/200 HCl	ACTIVATED SALIVA: Neutrality to phenolphthalein = cc. N/200 Na_2OH	ACTIVATED SALIVA: Neutralising power	Salivary factor
				Carious with care						*per cent*
C-27	July 12		July 12	18 85	3 35	22 20	22 80	1 40	24 20	91 73
C-27	July 14	light	during convulsion				4 30	27 80	32 10	
C-32	July 9	severe	July 12	24 95	2 45	27 40	26 65	1 30	27 95	98 03
C-32	July 14	light	during convulsion				2 10	26 30	28 40	

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taken during a quiet period. In the case of C-27 the *first* sample was taken five hours after a seizure and the *second*, two hours later *at the time of a seizure*. The acidity, in the first instance, of the activated saliva, is 1.40 cc. $\frac{N}{200}$ NaOH, and in the second has increased to 27.80 cc. In the case of patient C-32 the first sample was taken three days after a severe seizure. Two days later a *second sample was obtained during a convulsion*. In this instance also is noted a marked increase of acidity in the second sample. Coexistent with this increase there is a corresponding decrease in the alkalinity which tends to render constant, within the variation of the experimental error, the total neutralizing power of this activated sample.

In table 5 patients C-28, C-29, C-33, C-35, C-46, and C-53 all present the same characteristics as previously noted. Another interesting point brought out by these experiments is the fact that the saliva regained its "normality" in twenty to thirty minutes after all symptoms of the seizure subsided. Patient C-40, an example of this, is reported below. The patient wore artificial dentures and the clinical conditions, therefore, could not be determined.

The results of the analyses are as follows:

			NORMAL RESTING SALIVA				ACTIVATED SALIVA			
No. of patient	Date last recorded convulsion	Severity of convulsion	Time at which sample was secured	Neutrality to P-nitro phenol = cc. N/200 HCl	Neutrality to phenolphthalein = cc. N/200 NaOH	Neutralizing power	Neutrality to P-nitro phenol = cc. N/200 HCl	Neutrality to phenolphthalein = cc. N/200 NaOH	Neutralizing power	Salivary factor
C-40	July 13, 10 a.m.	Very severe	July 13, 3.30 pm	19.35	6.70	26.05	22.70	2.65	25.35	102.75
C-40	July 13, 5.50 p.m	Very severe	July 13, 6.15 p.m	19.40	4.30	23.70	26.70	−3.50 alk.	23.20	102.1

The second sample was secured *twenty minutes after* the seizure subsided. In this case the amount of saliva secreted at the *time of the convulsion* was too scanty to obtain a sample. This patient, a woman thirty-seven years of age, single, has been an epileptic for the past four years. It will be noted that the salivary factor varies less than 1 per cent which may be considered an unusually exact check for in the majority of cases the most careful analytical work will generally admit of no closer agreement than 5 per cent. The relatively high alkalinity to phenolphthalein, of the activated sample in second test, as shown

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TABLE 3

Epilepsy

No. of patient	Date last recorded convulsion	Severity of convulsion	Time at which sample was secured	NORMAL RESTING SALIVA: Neutrality to P-nitro phenol cc. N/200 HCl	NORMAL RESTING SALIVA: Neutrality to phenolphthalein = cc. N/200 NaOH	NORMAL RESTING SALIVA: Neutralising power	ACTIVATED SALIVA: Neutrality to P-nitro phenol cc. N/200 HCl	ACTIVATED SALIVA: Neutrality to phenolphthalein = cc. N/200 NaOH	ACTIVATED SALIVA: Neutralising power	Salivary factor
					Carious with ut care					
										per cent
C-28	July 9	light	July 12	25.95	8.70	34.65	43.40	− 2.00 alk.	41.40	83.70
C-28	July 13	light	during convulsion				3.25	35.10	38.35	
C-29	July 12	severe	2 hrs. after convulsion	23.15	3.15	26.30	22.55	2.50	25.05	105.00
C-29	July 12	severe	during convulsion				3.60	28.10	31.70	
C-33	July 12	severe	July 13	11.35	8.50	19.85	20.90	2.25	23.15	85.75
C-33	July 13	light	during convulsion				6.70	26.90	33.60	
C-34	July 12	severe	July 13	17.45	5.00	22.45	51.10	−3.70	47.40	46.36exc
C-35	June 12	light	July 13 9 a.m.	13.55	7.00	20.55	22.85	3.30	26.85	78.40
C-35	July 13	severe	during convulsion				1.60	27.00	28.60	
C-37	July 13	light	2 hrs. after convulsion	24.50	6.90	31.40	26.05	2.70	28.75	109.22

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C-38	July 10	severe	July 13	24.25	5.00	29.25	26.60	−0.60 alk.	26.00	112.50
C-41	June 1	severe	July 13	16.25	17.75	34.00	19.15	2.80	21.95	154.90
C-43	July 10	light	July 13	44.00	−4.00 alk.	40.00	36.20	−2.50 alk.	33.70	118.69
C-45	July 13	severe	2 hrs. after convulsion	16.10	4.75	20.85	22.45	−3.50 alk.	18.95	110.02
C-46	July 14	severe	2 hrs. after convulsion	14.75	5.85	20.60	12.20	3.55	15.75	130.80
C-46	July 14	severe	during convulsion				2.40	27.60	30.00	
C-51	July 13	severe	July 14	17.60	14.05	31.65	25.25	3.35	28.50	111.05
C-53	July 10	severe	July 14	28.70	6.00	34.70	37.60	−1.50 alk.	36.10	96.12
C-53	July 15	severe	during convulsion				7.00	26.20	33.20	
C-50	July 13	severe	July 15	27.15	2.55	29.70	34.25	1.35	35.60	83.43

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by the minus sign, does not markedly alter the relationship of the normal resting saliva to the activated saliva, for the neutralizing power, as determined separately has been maintained constant to within a relatively small variation.

Consider these data from a physiological standpoint. The muscular convulsions incident with an epileptic seizure of the severe type, referred to by Osler (2), as the grand mal, in contradistinction to the petit mal, in which the convulsions are very much less marked, increase the formation, in the tissue, of the oxidative resultant, namely, the paralactic acid. Halliburton (3) in discussing the chemistry of muscle during work says that "It (para-lactic or sarco-lactic acid) is the lactic acid par excellence of muscle. It is found also in the blood *especially after* muscular activity." Although some authors discuss at great length the probability of the proteid origin of lactic acid as for instance Bohm (4), Latham (5), Araki (6) and Hammersten (7) the consensus of opinion favors the glycogen theory. Both Halliburton (3) and Howell (8) assume that the stored glycogen of muscle is first converted to dextrose by the action of an amylolytic enzyme and then the dextrose is split according to following:

$$C_6H_{12}O_6 = 2C_3H_6O_3 \text{ and finally to } CO_2 \text{ and } H_2O$$

Since the genesis of the secretion of the salivary glands is in the blood (Halliburton) (9) it follows that the increased acidity of the saliva, produced during a seizure, is due to the corresponding increase of acidity in the blood resulting indirectly from the muscular work.

SUMMARY

1. That the neutralizing power of the saliva secreted by individuals suffering from dementia praecox bears a definite relationship to oral conditions.

2. That this relationship is the same as that observed in the normal individual.

3. That the neutralizing power of the saliva secreted by individuals suffering from epilepsy shows the same relationship to all conditions.

4. That during the time of muscular activity incident to an epileptic seizure the acidity of the saliva is markedly increased.

5. That the *alkalinity of saliva* produced during this period of stress is correspondingly lowered thus holding constant its total neutralizing power.

6. That the normality of the saliva is regained within thirty minutes after symptoms of the seizure have subsided.

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THE EFFECTS OF THE SUBCUTANEOUS INJECTION OF ORGAN EXTRACTS UPON THE FLOW OF PANCREATIC SECRETION

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In our last communication upon the physiology of organ extracts(1) we summarized the results of our previous experiments. We found that the non-coagulable, or residue, portion of the aqueous extract of certain organs seemed to contain all of the material which showed demonstrable physiological activity. The residues of the pituitary, pineal, thyroid, parathyroid, thymus and adrenal glands, and of the liver, spleen and pancreas seemed each to exert a characteristic effect upon the blood pressure, respiration and heart action (2) and upon the contraction of unstriated muscle fibre (3). The action of the residues upon unstriated muscle fibre seemed to be exerted through the terminal filaments of the nerve supply of the muscles. The residue or non-coagulable portion of some of these extracts seemed also to have a more or less specific effect upon the gastric secretion, and the thyroid residue proved in addition to be a vigorous stimulant of gastric peristalsis (1).

The following communication deals with the effects of the coagulable and non-coagulable, or residue, portions of aqueous extracts of organs upon the flow of pancreatic secretion. Dogs were employed for the tests, and females were found, as a rule, to be more easily handled than males.

The lower and larger pancreatic duct, or that of Wirsing, was cut out of the wall of the duodenum, together with a small portion of the surrounding gut, and sutured into a median laparotomy wound. After healing had ensued, the dog was placed in a frame, and the flow of pancreatic juice from the fistula was estimated by counting the drops during five minute periods. The normal average flow per minute was thus determined during fifteen minutes. No food was given to the

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animal for twelve hours before the experiment and none immediately afterwards.

The test material was standardized according to its nitrogen content, and equal amounts were injected aseptically into the subcutaneous tissue of the back or loin.

After having determined the normal flow, the injection of the test material was made and the drops from the fistula during many succeeding five minute periods were counted and recorded as in the table. Some ten or a dozen animals were employed and the table which give the record of an individual animal is typical of the results obtained.

In the course of these experiments it was apparent that external conditions or impulses, which presumably originating in the central nervous system, had a considerable influence upon the rate of flow from the fistula. Any excitement of the animal seemed to check the discharge immediately. This factor made the experiments somewhat unsatisfactory, but the results appeared to be of sufficient interest to warrant their publication. It is well recognized that an increase of the gastric secretion, and especial, of its hydrochloric acid, stimulates the flow of pancreatic secretion. In our last communication in this series (1) we demonstrated that the residues or non-coagulable portions of an aqueous extract of the thyroid and parathyroid glands and of the liver, pancreas and spleen increased both the amount and the acidity of the gastric secretion. In the following table it will be noted that the residue of the parathyroid gland and of the spleen and pancreas, although they stimulate gastric secretion, do not stimulate that of the pancreas. The thymus residue did not appreciably increase flow of gastric secretion, but was a most vigorous stimulant of the flow from the pancreas. The liver residue proved to be a most active stimulant for both the gastric and pancreatic secretion. But it stimulates the pancreas so soon after the injection that the effect seems the result of a direct action rather than through the increase of a secretion of hydrochloric acid.

The delayed stimulant effect of the thyroid residue suggests that it may be indirect, and secondary to the gastric mechanism. On the other hand the similar delayed effect of the thymus residue which does not appreciably increase the gastric secretion suggests that the thymus residue may act directly upon the pancreas. The adrenal residue inhibits the flow of the pancreatic secretion. The effects of the adrenal residue are approximately the same as those of the usual 1: 1000 solution of adrenalin chloride.

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		BROPS IN 1ST 5 MINS.	BROPS IN 2ND 5 MINS.	BROPS IN 3RD 5 MINS.	BROPS IN 4TH 5 MINS.	BROPS IN 5TH 5 MINS.	BROPS IN 6TH 5 MINS.	BROPS IN 7TH 5 MINS.	BROPS IN 8TH 5 MINS.	BROPS IN 9TH 5 MINS.	BROPS IN 10TH 5 MINS.	BROPS IN 11TH 5 MINS.	BROPS IN 12TH 5 MINS.	BROPS IN 13TH 5 MINS.	BROPS IN 14TH 5 MINS.	BROPS IN 15TH 5 MINS.	BROPS IN 16TH 5 MINS.	BROPS IN 17TH 5 MINS.	BROPS IN 18TH 5 MINS.	BROPS IN 19TH 5 MINS.	BROPS IN 20TH 5 MINS.	AVERAGE PER MIN.	
Thyroid residue	Normal	5	5	4	3	3	2	2	1	4	3	6	17	35	37	36	38	37	38	34	29	30	1.0
	After injection		4	7	8	3	2	2	1	4	3	6	17	35	37	36	38	37	38	34	29	30	3.8
Thyroid coagulable	Normal	8	7	7	3	2	2	1	5	7	3	7											1.5
	After injection	1	14	13	12	9	12	15	27	27	11	11	8	0	4								2.1
Liver residue	Normal	6	11	12																			2.7
	After injection		15	17	25	27	32	37	48	52	50	50	50	47	55	54	52	64	43	30	41		7.1
Liver coagulables	Normal	11	9	13																			2.2
	After injection		16	25	11	9	11	5	7	3	7												2.1
Thymus residue	Normal	11	17	10																			2.5
	After injection	8	8	7	8	11	11	27	31	31	30	48	49	47	40	34	43	38					5.9
Thymus coagulables	Normal	21	19	20																			4.0
	After injection	17	16	14	13	10	12	13	12	12	11	7											2.5
Adrenal residue	Normal	10	10	10	6																		1.8
	After injection	3	24	14	5	2	2	2	1	2	2	1	1	1	1								0.9
Pancreas residue	Normal	22	16	15	13	13	21	17	14	7	8	7	5	4									3.5
	After injection	10	15	16	17	12	12	7	7	8	6	7											2.0
Spleen residue	Normal	13	15	14																			2.8
	After injection	10	9	4	3	13	17	22	20	5	4												2.3
Pituitary residue	Normal	26	21	23																			4.6
	After injection	7	6	15	13	15	22	16	18	16	19	20											3.0
Parathyroid residue	Normal	15	25	12	11	11																	3.0
	After injection	12	10	9	7	6	10	13	3	13	9	12											2.1
Adrenalin	Normal	26	24																				5.0
	After injection	30	10	3	1	2	3	1	3														1.3
Effects of feeding	Normal	11	10	10																			2.0
	Three hours later	44	34	26	31																		6.0

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All of these results agree with those previously obtained in localizing the active principles of any organ in the non-coagulable or residue fraction portion of its aqueous extract.

CONCLUSIONS

1. The effect of the subcutaneous injection in dogs of the residue, or non-coagulable portion, of an aqueous extract of the liver is the immediate and vigorous stimulation of the external secretion of the pancreas.

2. The residues of the thyroid and thymus produce a somewhat less vigorous and later response.

3. The residues of the pituitary and parathyroid glands and of the spleen and pancreas are inert.

4. The residue of the adrenal gland, like adrenalin, vigorously inhibits the intestinal secretion of the pancreas.

5. Only the residue or non-coagulable portion of an aqueous extract of the above mentioned organs shows any appreciable effect upon the intestinal secretion of the pancreas.

6. From these, and from the tests previously reported, we conclude that the residue or non-coagulable portion of an aqueous extract of the pituitary, pineal, parathyroid, thymus and adrenal glands and of the liver, spleen and pancreas contains practically all of the material from each of these organs which can directly and immediately affect through the circulation the functional activity of any other organ.

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ACCELERATION OF GROWTH AFTER RETARDATION[1]

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In studying the curves of growth of a considerable number of albino rats in which, for a diversity of reasons, growth had been inhibited for varying periods, we have been impressed by the unexpectedly accelerated rate at which the increment of body weight may be resumed when the conditions are favorable. The circumstances preceding the renewal of growth in these instances were not directly comparable with those determining the rapid increase in weight that follows considerable depletion of the body substance. We have already published illustrative charts showing "curves of repair" after a considerable decline in body weight due to feeding with a defective diet (1). They indicate that under suitable dietary conditions lost weight may be regained far more rapidly than during normal growth through the same range of body weight. It was pointed out in our earlier publication that the chemical or metabolic processes of repair are probably by no means identical with growth. They may not involve the destruction and resynthesis of an entire protein molecule or of the entire protoplasmic cell structure. It is, furthermore, a familiar fact that repair or recuperation can take place at all ages, even after the completion of ordinary growth in the individual. The series of observations at present under consideration concern the weight changes subsequent to a more or less prolonged interruption of growth without any significant decline in body weight.[2]

[1] The expense of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

[2] Rat 2523 ♂ forms an exception in that it had declined 78 grams from a body weight of 263 grams at a period considerably before the resumption of growth. The first rapid increase in weight in this animal thus in part includes a period of "repair."

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It has been shown that the capacity to grow can be retained and exercised at periods far beyond the age at which growth ordinarily ceases (2). There seems to be no necessary impairment of the individual with respect to the ability subsequently to reach the full size characteristic of the species. Already in an earlier paper (2) it was noted that the rate at which growth is resumed after these prolonged delays need not be slow, and frequently actually exceeds the usual progress.

Anomalies of growth expressed by an exaggerated rate of growth are among the rarities of physiology. In referring to instances in the pathology of infancy Schloss writes:

> Was von excessiver Massenzunahme dem Kliniker häufiger vor Augen kommt, sind die Fälle, bei denen nach vorausgegangenem längeren Gewichtsstillstand, sei es durch Inanition, sei es durch Störungen der Entwicklung infolge von äusseren Schädigungen, das versäumte Wachstum schnell nachgeholt wird. Aber hier ist es fraglich, ob es sich um wirkliches Neuwachstum, also um eine enorme Beschleunigung der Zellteilung und Massenablagerung handelt; wahrscheinlich ist während des vorausgegangenen Entwicklungsstillstandes ein grosser Teil der Wachstumsarbeit schon getan, so dass nur noch die stoffliche Ausfüllung des Vorgebildeten übrig bliebe. In dem letzteren wahrscheinlicheren Fall gehörte diese Art 'Nachwachstum' zu den progressiven Korrelationsstörungen (3).

In other words whenever the growth of an entire organism as well as that of individual organs is modified in the sense of acceleration, this usually involves the reversal or return of a morbid condition to the normal. Further indications of the wide-spread uncertainty regarding the effects of retardations of growth have been expressed by Rubner, as follows: "We really do not know whether nature demands an absolutely uniform daily growth or whether remissions are permissible or perhaps even advantageous (4)."

In the case of children Boas (5) has reported that retarded individuals possess a late acceleration of growth. His statistical analyses show "that individuals whose prepubertal accelerated growth begins late in life have rates of growth that exceed by far those of the normal individual; in other words, that among the retarded individuals the whole energy required for growth is expended in a very brief period." Boas adds: "It seems very likely that the abnormally large amount of energy expended upon rapid growth during a short period is an unfavorable element in the individual development."

Schapiro (6) has found that if young kittens were chloroformed twice a day their growth was retarded in comparison with normal control

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animals. However, on stoppage of the chloroform treatment, the greater rapidity of growth during an after period fully compensated for the earlier delay in development.

Quite recently Stewart (7) has reported the results of refeeding upon the growth of the body and of various organs of young albino rats after inanition for various periods. The growth in body weight of the rats refed after maintenance for various periods averaged considerably higher for some time than the normal for (younger) controls of the same body weight. Thus the stunted rats on refeeding were able to overtake the full-fed controls before the end of the normal growth period.

The accompanying tabular summary presents statistics of the accelerated growth which we have observed in illustrative instances. The "normal" figures for the average body weight at different ages and sexes in albino rats are taken for comparison from the most recent compilation of Dr. King (8) at the Wistar Institute. The retardation of growth was brought about in a variety of ways in the individual animals: sometimes intentionally, by the character of the diet fed; sometimes incidentally as the result of a failure on the part of the animals to eat enough of a supposedly adequate ration. Only those increments of size beyond the maximum weight previous to the resumption of growth are included in the figures recorded.

A few typical records are further illustrated in the appended graphic charts. These, as well as the summarized data, are practically self explanatory. An illustrative case may, however, be cited in detail to emphasize some of the points involved. Rat 2339 ♀ (see Chart I Appendix), for example, was maintained for some time without growth at a weight of about 100 grams until the late age of 402 days. At this age female rats (according to Dr. King's averages) weigh 220 grams. Growth was now resumed at an enormously exaggerated rate, so that *the rat put on 112 grams* and reached a weight of 216 grams *in 26 days*. The normal growth from 104 to 216 grams in female rats ordinarily requires 233 days. The record of Rat 2598 ♂ (see Chart II, Appendix) likewise may be cited, from among many others. It shows *a gain of 150 grams in body weight in 36 days* at a size which normally requires considerably more than two hundred days for the same growth accomplishment. These are merely typical cases. It will be observed that where the resumption begins early enough, these rapidly growing animals overtake the average growth record before growth is completed. There is also a tendency to reach a size decidedly larger than the average for this species.

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Summary of changes in body weight in albino rats showing an accelerated rate of growth after retardation

RAT	AGE AT WHICH GROWTH WAS RESUMED	BODY WEIGHT WHEN GROWTH WAS RESUMED	AVERAGE NORMAL WEIGHT FOR THIS AGE	AVERAGE NORMAL SIZE REGAINED AT		TIME REQUIRED TO MAKE THE RECORDED GAIN IN BODY WEIGHT	
				Body weight	Age	In this experiment	In normal growth
♀	*days*	*gm.*	*gm.*	*gm.*	*days*	*days*	*days*
2446	426	198	216	218	438	12	86
2339[1, 6]	402	104	220	216	428	26	233
2369[1]	380	102	222	218	438	58	242
2476[1, 6]	322	116	221	220	400	78	244
1127[1, 3]	190	144	196	208	238	48	150
♂							
2523[2]	399	263	314	313	413	14	199
2562[2]	376	272	309	314	399	23	171
2520	352	225	304	310	394	42	258
2448[2]	350	290	304	304	354	4	56
2447[2]	348	236	303	309	371	33	238
2609	315	261	298	302	347	32	148
2611	312	249	298	301	363	51	171
2180[2, 6]	303	73	296	324	459	156	265
2549[2]	293	170	290	299	349	56	237
2598[2]	249	130	280	287	285	36	221
708[2, 5]	203	157	266	280	263	60	167
2293[2]	193	69	262	305	359	166	320
2911[2]	188	170	243	267	206	18	123
1568	157	117	248	280	257	100	185
2030	152	183	245	256	176	24	87
1204[2, 4]	145	129	241	272	224	79	161
1180[2]	136	133	234	272	223	87	158
1276[4]	112	133	211	260	188	76	123
1236	111	104	210	265	202	91	149
1076	83	90	170	253	168	85	121

[1] See Chart I, Appendix.
[2] See Chart II, Appendix.
[3] See Chart VI, Jour. Biol. Chem., 1913, xv, 325.
[4] See Chart I, Ibid., xvi, 433.
[5] See Chart I, Ibid., 1914, xviii, 104.
[6] See figure 5, Ibid., 1915, xxiii, 454.

Although these curves of "resumed growth" are usually far more "steep" than the normal curve of growth appears at any stage of its progress, the actual *percentage* increment is nowhere as large as is found in the earliest period of normal growth (9). Nevertheless the power of growth measured by the percentage rather than absolute increments of weight is decidedly greater than is ordinarily noted at the same

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size in uninterrupted growth. The daily increments during "resumed growth" after the age of 100 days may equal 4 per cent, whereas normally they rarely exceed 1 per cent.

The ability of the individual to make exceptionally rapid gains of weight after a period of enforced maintenance without growth raises questions of broader biological interest. What has time accomplished in the interval of unchanged total body weight? Have developmental changes or cellular rearrangements proceeded? Have some of the cells (perhaps those of certain endocrine glands) advanced in their development more nearly normally than the great mass of the tissues? If so, they might exert an undue stimulus upon the energy transformations leading to growth. Stewart (7) has pointed out that there are variations in the relative weights of different viscera during maintenance without growth. The weight of some (including the hypophysis, testes and suprarenals) is even said to increase under such conditions. The explanation of the recorded phenomena of an exaggerated rate of growth after suppression of growth calls for elaborate histological studies of the important tissues during these periods of exceptional change.

SUMMARY

Records are presented to show that *after periods of suppression of growth*, even without loss of body weight, *growth may proceed at an exaggerated rate* for a considerable period. This is regarded as something apart from the rapid gains of weight in the repair or recuperation of tissue actually lost. Despite failure to grow for some time the average normal size may thus be regained before the usual period of growth is ended.

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EVIDENCE THAT THE ACTIVE PRINCIPLE OF THE RETRO-PERITONEAL CHROMAPHIL TISSUE HAS THE SAME PHYSIOLOGICAL ACTION AS THE ACTIVE PRINCIPLE OF THE SUPRARENAL GLANDS

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INTRODUCTION

From the large amount of work done on the chromaphil system, Vincent (1) concludes that all chromaphil tissue, whether contained in the suprarenal gland or not, yields adrenin, or a substance having a similar pharmacodynamical action, and states that this conclusion is based upon the provisional assumption that chromaphil tissues are specific in their nature, and everywhere of the same essential character. In support of this hypothesis Biedl and Weisel have shown that extracts of the retro-peritoneal tissues in man have the same effect on arterial blood pressure as extracts of the suprarenal glands. The hypothesis has not, however, been tested by the more exact methods which have been developed during recent years, and it was with this object in view that the present investigation was undertaken.

METHODS

The pharmacodynamical tests employed have been those advocated by G. N. Stewart (2), namely, the action on the spontaneous contractions of the isolated intestinal muscle and on the tone and contractions of the virgin uterus of the rabbit. An inhibition of the former, along with an augmentation of the latter, was taken as positive evidence of the presence of epinephrin. As was shown by Stewart, the occurrence of these opposite effects entirely removes any doubt which might otherwise be raised when one physiological action alone is employed; it eliminates any confusion that might arise on account of the presence of proteins or other pressor or depressor substances in the extracts.

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Two similar muscle chambers of glass, one with a capacity of 50 cc., the other of 30 cc., were used for the intestinal and uterine preparations respectively. The lower end of the muscle chambers extended downwards through the vessel used as a waterbath. Two small capillary glass tubes were fastened in the lower end of each of the muscle chambers. One of the tubes was connected by rubber tubing to the oxygen tank. Through this tube very small bubbles of oxygen were allowed to escape into the solution. The other tube served as an outlet for withdrawing the solution contained in the chamber. In this way it was possible to change the fluids in the chambers with very little mechanical interference with the action of the muscle preparations. The preparations of intestine and uterus were quickly removed from the same (non-pregnant) rabbit.

The difficulty of detecting epinephrin in extracts of the retro-peritoneal chromaphil tissue is greatly enhanced by the fact that the protein of these extracts exerts a marked influence on the movements of the intestine and uterus. Protein causes a great increase in the tone of both muscle preparations. In the case of the intestine the epinephrin inhibition must overcome the pressor effects of the proteins present before it can manifest itself. In the case of the uterus, epinephrin causes an augmentation and acceleration of the uterine muscle; so also does protein.

In obtaining the retro-peritoneal chromaphil tissue for the physiological test, an attempt was made to get as much of the tissue as possible. The suprarenal glands were first carefully dissected out. The kidneys were next excised and discarded, care being taken to cut the blood vessels as near the organ as possible, as the chromaphil tissue is regularly found in the renal plexus. A small piece of tissue about a centimeter in length was next removed to serve as a histological check. The remainder of the abdominal aorta and surrounding tissues was carefully dissected out. The suprarenal glands and this retro-peritoneal tissue, with the exception of the above histological check, were, upon excision, washed free from blood and placed in the extracting solution.

In the earlier attempts the extracting solution consisted of Ringer's solution, but it was soon found that the larger percentage of protein and the rapid oxidation of epinephrin rendered the results very uncertain.

The method that was found to eliminate the source of error most satisfactorily was a modification of that which Folin, Cannon and

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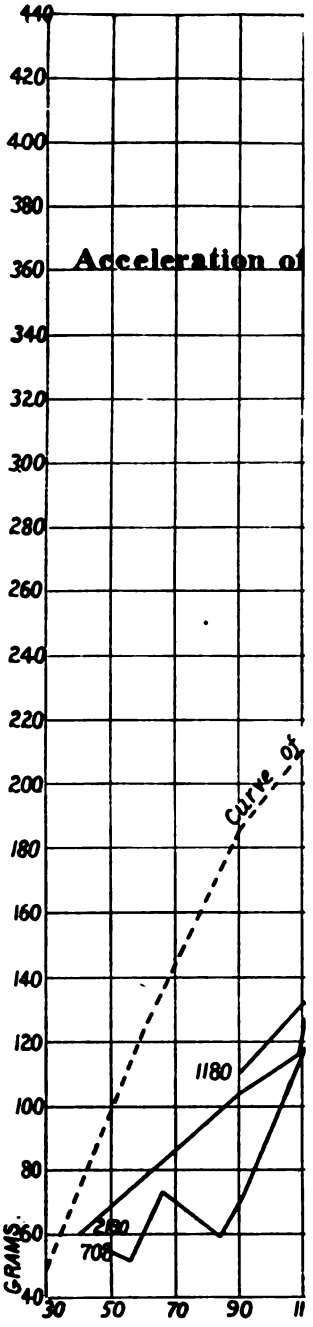


CHART II. Chart showi
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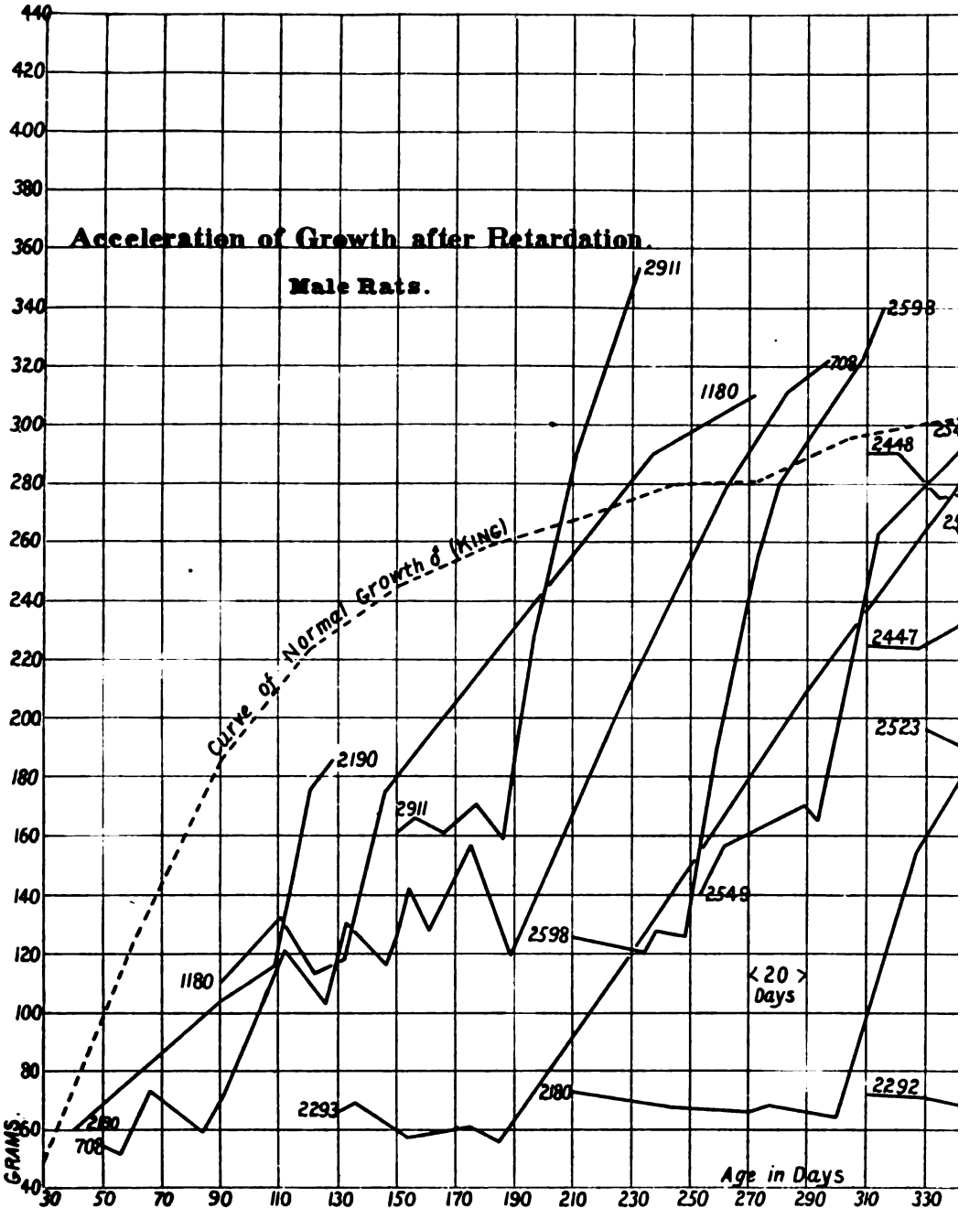


CHART II. Chart showing the increments of body weight in male albino rats after periods of or completely suppressed. Growth was resumed in these animals at an accelerated rate fa comparable size under normal conditions of uninterrupted growth. Statistics for some of thes the text.

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Denis (3) employed for the chemical assaying of the epinephrin in the suprarenal glands. The suprarenal glands and the retro-peritoneal tissues were put into separate beakers containing 10 cc. of a $\frac{N}{10}$ HCl solution plus a few cubic centimeters of distilled water. The tissue was then finely minced and macerated with sand in a mortar, after which the extract was transferred to a beaker with the addition of a few cubic centimeters of water and slowly brought to boiling. The extract was then expressed through surgical gauze to separate the sand and larger parts of tissue from it. The precipitate was again extracted with 5 cc. of $\frac{N}{10}$ HCl and a few cubic centimeters of distilled water. The filtrates were then poured together and the total volume of extract brought to 40 cc. by the addition of more distilled water. The total extract was again brought to boiling, when 5 cc. of a 10 per cent solution of sodium acetate was added and the boiling continued for a few minutes, after which the material was filtered. The extract of each tissue was then transferred to stoppered flasks in the waterbath until ready for use. The best results were obtained by neutralizing the extract (towards litmus) with a 10 per cent solution of sodium carbonate before applying it to the muscle preparation.

RESULTS

For convenience the results are presented in tabular form (table 1) a few of the curves from typical experiments being also given in figures 1, 2 and 3. It will be unnecessary here to do more than refer to the experiments in which the technique was modified, or the results obtained were out of the usual run, or refer to tissue in which some doubt has hitherto existed as to the presence of chromaphil tissue.

Regarding the mode of preparation and the optimum reaction of the extract, it was found that in extracts prepared by means of Ringer's solution from the tissues of a dog, those of the suprarenal glands caused inhibition of the movements of the intestinal muscle and augmentation and acceleration of the movements of the uterine muscle, whereas similar extracts of the retro-peritoneal chromaphil tissue gave an augmentation followed by a slight diminution of the movements of the intestinal muscle and an augmentation followed by a diminution of the uterine muscle, both reactions being probably due to protein, since similar extracts of the thoracic aorta and muscle tissues gave corresponding results. It was concluded that the epinephrin had oxidized during the process of extraction to such an extent that the inhibitory

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effect of the epinephrin could not overcome the pressor effect of the proteins present.

In all the other observations on the dog, the method of acid extraction was employed and, as shown in the table, distinct evidence of epinephrin

TABLE I

NO.	ANIMAL INVESTIGATED	TISSUE EMPLOYED	HISTOLOGICAL CHECK	ACTION ON INTESTINE	ACTION ON UTERUS
1	Dog*	Retro-peritoneal	+	−	−
		Suprarenals		+	+
2	Dog	Retro-peritoneal	+	+	+
		Suprarenals		+	+
		Thoracic aorta		−	−
		Muscle		−	−
		Muscle + epinephrin		+	+
3	Cat	Retro-peritoneal	+	+	+
		Suprarenals		+	+
		Thoracic aorta		−	−
4	Dog	Retro-peritoneal		+	+
		Suprarenals		+	+
		Muscle		−	−
5	Rabbit	Retro-peritoneal	+	+	+
		Suprarenals		+	+
6	Guinea pig	Retro-peritoneal	+	+	+
		Suprarenals		+	+
7	White rat	Retro-peritoneal	+	+	+
		Suprarenals		+	+
8	Dog	Retro-peritoneal		+	+
		Suprarenals		+	+
		Thoracic aorta		−	−
9	Cat	Retro-peritoneal	+	+	+
		Suprarenals		+	+
10	Calf	Retro-peritoneal		+	+
		Suprarenals		+	+
11	Pig	Retro-peritoneal		+	+
		Suprarenals		+	+
12	Sheep	Retro-peritoneal		+	+
		Suprarenals		+	+
13	Child	Retro-peritoneal		−	−
		Suprarenals		−	−
14	Man	Retro-peritoneal		+	+
		Suprarenals		+	+

*Extracts prepared by Ringer's solution.

+ Under "Action on intestine" and "Action on uterus" indicates positive evidence of epinephrin.

− Indicates no epinephrin reaction.

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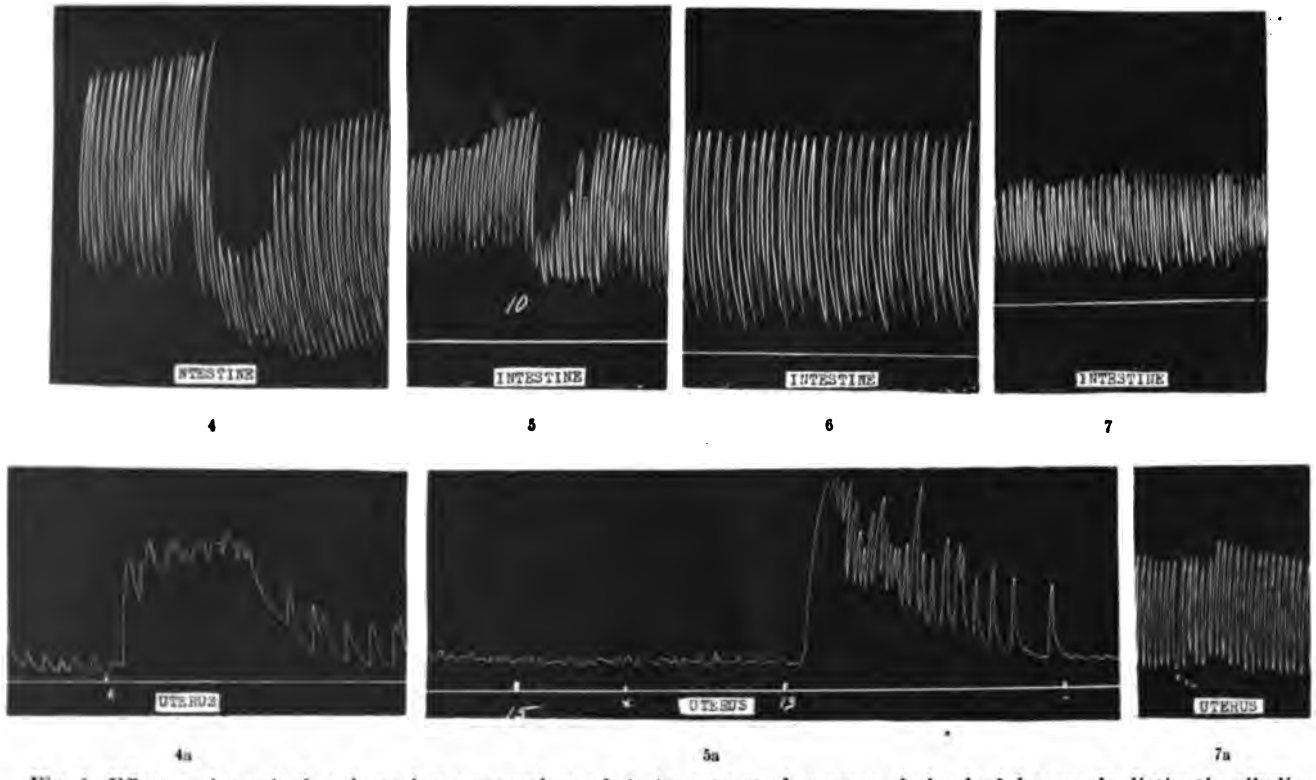


Fig. 1. Effect on intestinal and uterine contractions of: *4*, *4a*, extract of suprarenal gland of dog, made distinctly alkaline to litmus; *5*, *5a* (13), extract of retro-peritoneal tissue of dog, neutral to litmus; *6*, *5a* (15), extract of thoracic aorta; *7*, *7a*, extracting solutions alone.

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was obtained in the retro-peritoneal tissue. In similar extracts prepared from other tissues, such as connective tissue, thoracic aorta, or muscle, no such results were obtained. Carefully prepared extracts of the sympathetic chain of ganglia and of the stellate ganglia, removed from several dogs, failed to give any indication of epinephrin. The result with the stellate ganglia is of interest, since chromaffin cells

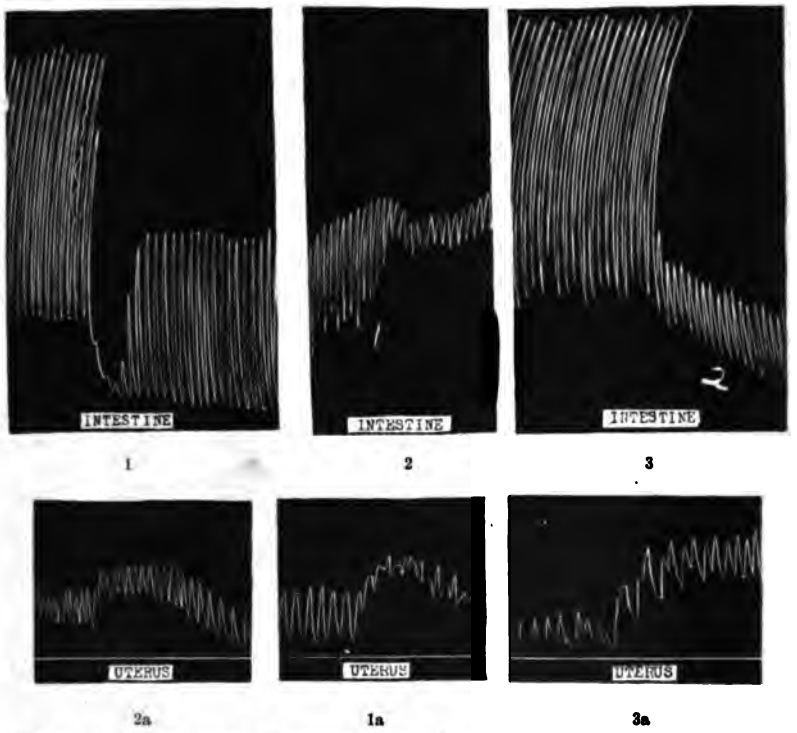


Fig. 2. To demonstrate the importance of using weak acid instead of Ringer's solution in preparing the extracts. *1*, *1a*, extract of suprarenal gland made by Ringer's solution; *2*, *2a*, extract of retro-peritoneal tissue of dog made by Ringer's solution; *3*, *3a*, extract of retro-peritoneal tissue of dog made by weak acid.

have been found present in them. Observations were made on the epididymis, in which such cells are also said to be present with negative physiological results. The most striking results were obtained when the solutions were as nearly neutral to litmus as possible (4). They were, however, left very slightly acid in order to prevent oxidation of the epinephrin.

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Special interest attaches to the observations on the white rat, the guinea-pig and man. By the macroscopic method of Kohn, no chromaffin tissue can be demonstrated in the retro-peritoneal tissue of the white rat and guinea-pig, although one of us (M. E. F.) has found it in this position by the use of the histological method. Extracts of these tissues gave strongly positive reactions with the uterine and intestinal muscle (fig. 3) preparations, thus confirming the conclusion that retro-peritoneal chromaffin tissue is present in these animals.

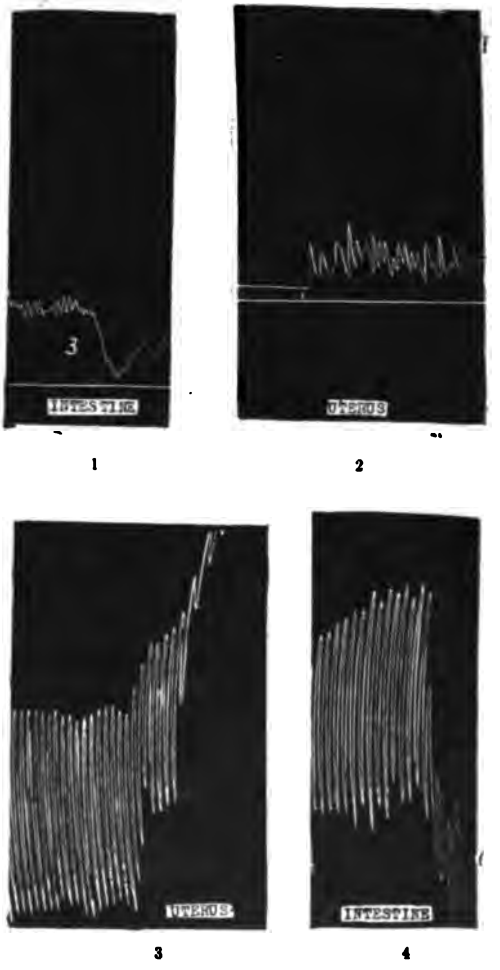


Fig. 3. To demonstrate effect of acid extract of retro-peritoneal tissue of white rat (*1* and *2*) and guinea pig (*3* and *4*) on intestine and uterus preparations.

The retro-peritoneal tissue obtained from a child three weeks old, which had died six hours after operative procedure for congenital hypertrophic stenosis of the pylorus, was after four days' time treated in the usual manner. The results obtained were negative, probably because of oxidation of the epinephrin in the tissues used. In the case of the retro-peritoneal tissue of a man, the results were positive. The subject used for this experiment was forty years old, mature, well-built and healthy. He was injured in an automobile accident and died of concussion of the brain four days after the injury. Five hours after his death, the retro-peritoneal tissue and suprarenal glands were excised and placed in the HCl solu-

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tion. The tissues were immediately extracted and tested, and as stated above positive results were obtained.

In two instances where very dilute solutions of the retro-peritoneal tissue extract were added to the uterus, a purely inhibitory effect was observed; five times the same dose which gave this effect gave the usual augmentory result. Stewart (2) observed that this was true of epinephrin in very dilute solutions. In three instances when the extracts of retro-peritoneal tissue were added to the intestinal preparations, an augmentation and acceleration were noted. Increased doses of the same extract produced the usual effect. Whenever these opposite effects were noted, the muscle preparations were always immediately washed with Ringer's solution and allowed to return to normal, when increased doses were applied. These results are in conformity with those of Cannon, Stewart, Hoskins, etc., who observed that very dilute solutions of epinephrin produce augmentory effects on the intestinal muscle. Similar reversed effects of epinephrin had previously been noted by Moore and Purinton (5) in the action on the blood pressure, a fall instead of a rise being obtained when very small doses of epinephrin extracts were administered. Pari (6) found that in freshly prepared extracts there is never a lowering of blood pressure, but that with very dilute solutions which have been kept for some time, this may be observed. He suggests, therefore, that the depression is due to a chemical change in the epinephrin. The fact that we observed opposite effects for epinephrin on the muscle preparations when dilute solutions were added, and that the normal effects were observed after increased doses, shows that the above explanation cannot be accepted.

In some of the records made by the uterine muscle, it will be observed that the muscle was recording a straight line before the addition of the solution to be tested. It appears that a muscle which is quiescent and records a straight line, may be just as irritable to epinephrin as its companions which are recording small contractions. Usually, however, the active strips furnish the best test objects.

Histological checks were made on the dog, the cat, the rabbit, the white rat and the guinea-pig. The yellow staining bodies and cells were demonstrated in all of the checks taken from the retro-peritoneal tissue.

We are indebted to Prof. T. W. Todd for the sectioning and staining of the histological checks in connection with this work, and to Prof. H. T. Karsner for the human material.

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CONCLUSION

Acid extracts of the retro-peritoneal chromaphil tissue of man, the dog, the cat, the rabbit, the guinea-pig, the white rat, the calf, the sheep, and the pig have the same physiological action on intestinal and uterine muscle as the active principle of the medulla of the suprarenal glands.

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THE REACTIONS OF STRIATED MUSCLE TO POTASSIUM CHLORIDE SOLUTIONS

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Since Overton pointed out that frog's striated muscle temporarily loses its irritability in solutions of potassium salts, and that these salts can be divided into two classes, according to the different effects which they have on muscle immersed in them (1), it has been shown by Siebeck (2) and by one of us (3) that Overton overlooked some important points regarding the reactions of muscle to potassium chloride. We agree with Overton in finding that frog's striated muscle maintains its original weight in isotonic solutions of dipotassium phosphate, and swells fairly rapidly in isotonic solutions of potassium chloride. But we disagree with him in regard to the permanently toxic effects of potassium chloride. It has been shown by Siebeck in the article referred to above that immersing a frog's sartorius in Ringer's solution may restore its original weight and irritability after it has been for four hours in 0.89 per cent potassium chloride solution at 0 to 4°C. In an experiment by one of us (Experiment 74 of the article cited above), a sartorius was kept for three hours in isotonic potassium chloride, and then for about twenty hours in Ringer's solution, the temperature throughout remaining near 16°. During the period in potassium chloride the muscle gained a little more than 30 per cent of its original weight and became, as was to have been expected, entirely unirritable. During the subsequent period in Ringer's solution, the muscle returned to a little less than its original weight and became as irritable as it was at the beginning. It is evident, therefore, that frog's sartorii may recover from the effects of an immersion in an isotonic potassium chloride solution.

The reactions of striated muscle to solutions of potassium salts are interesting from a number of different aspects, which need not be dwelt on here, and we have thought it advisable to try to gain some further light on the subject.

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PRELIMINARY EXPERIMENTS

We began by trying experiments in which frog's sartorii were immersed for various periods in 0.9 per cent potassium chloride solutions at between 5 and 10°, and then transferred to Ringer's solution. Experiments 1, 2 and 3 are examples of these. They show that recovery from the potassium chloride effects may be quite complete after six hours' immèrsion; and that, even after a sartorius has been for twenty-four hours in the potassium chloride solution and increased in weight 100 per cent, it may still show some degree of recovery. It must be added, however, that the results of such experiments are very variable. In experiments similar to nos. 1, 2 and 3, but carried out later in the summer, we failed to get recovery after exposures to potassium chloride of much less than twenty-four hours. We are inclined to attribute these differences to changes undergone by the frog's tissues with the advance in the season.

ANALYSIS OF THE POTASSIUM CHLORIDE EFFECTS

The experiments to be reported under this heading may be regarded as a preliminary attempt to analyze the changes which go on in a frog's sartorius when it is immersed in a 0.9 per cent potassium chloride solution. It is obvious that the muscle takes up water, and very probable that it takes up potassium chloride. And these changes are probably accompanied by still others which are not quite so obvious, but still highly important for an understanding of the phenomena. In the following paragraphs the experiments bearing on the various changes, either known or suspected to occur, are reported seriatim.

Does muscle immersed in potassium chloride produce lactic acid? It is now well recognized that striated muscle produces considerable quantities of lactic acid when subjected to a great variety of abnormal conditions, and the question comes up whether or not immersion in potassium chloride solutions may have this effect. There are many reasons for thinking that this is the case. It is well known, for instance, that the chloride, as well as other salts of potassium, cause muscle to go into an abnormal form of contraction which is maintained for some minutes, and all the forms of contraction and rigor so far investigated have been shown to be accompanied by lactic acid production. The influence of temperature on the reactions of muscle to potassium chloride confirm the supposition under discussion. In order to show the recovery from the potassium chloride effects, Siebeck carried out his

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experiments at between 0 and 4°. The experiments of one of us in the article referred to above show that the recovery from the potassium chloride effects is dependent on temperature. A muscle kept for six hours in 0.9 per cent potassium chloride solution at between 20 and 21° failed to recover its irritability, while another muscle kept for the same period in the same solution at between 2 and 8° did recover its irritability, very completely (4).

Another point, which indicates that the swelling undergone by muscle in a potassium chloride solution may be partly the result of lactic acid production by the muscle, is the interesting fact brought out by Siebeck, that sartorii swell faster in potassium chloride and recover less completely, if they be subjected to strong stimulation soon after their immersion (5). We have repeated this experiment and obtained similar results. We have found, however, as Siebeck implies, that in order to produce constant results the stimulation must be very strong—of such strength as would almost undoubtedly injure a normal sartorius. We found that more moderate stimulation[1] produced no constant effects either on the rate of swelling of muscle immersed in the potassium chloride solution, or on its subsequent tendency to recover its irritability.

The part played by lack of calcium in the potassium chloride effects. So far as we know, there is no solution of a single salt in which surviving muscle remains alive for any great length of time. A 0.6 per cent or 0.7 per cent sodium chloride solution is as little injurious as any other so far discovered, but in this solution an irritable muscle usually twitches continually, gains weight, and loses its irritability in less than twenty-four hours at ordinary room temperatures. Very small quantities of a soluble calcium salt added to the pure sodium chloride solution suffice to prevent the twitching and to greatly prolong the period during which the muscle remains irritable. It is possible, therefore, that the death of muscle in potassium chloride solutions may be due, in part, at least, to the absence of calcium, as well as to the direct effects of the potassium. We have tried experiments in which we have compared the results of

[1] As "moderate stimulation" we used a tetanizing current which could be borne without much discomfort on the tongue. Such a current would strongly stimulate a normal muscle, but would produce comparatively little injury; the individual shocks of which it is composed would be equivalent to from 400 to 1000 of Martin's Z units. As "strong stimulation" we used a current whose individual shocks would be equivalent to from 4000 to 8000 of Martin's Z units. See Martin: The Measurement of Induction Shocks; New York and London, 1912. We do not think it worth while to publish the protocols of these experiments.

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immersing sartorii in pure 0.9 per cent potassium chloride solution with those of immersing them in mixtures of this with 1 per cent calcium chloride solutions.[2] The mixtures were made by adding 97.5 parts of the potassium solution to 2.5 parts of the calcium solution, and were, therefore, very nearly isotonic with the original potassium solution. It was found that the muscles gained weight more slowly in the potassium and calcium mixtures than in the pure potassium solutions, and that they recovered their irritability more completely when subsequently immersed in Ringer's solution. See Experiments 4 and 5.[3]

It is a tempting hypothesis that the calcium produces its effects by rendering the surfaces of the muscle fibers less permeable to the potassium chloride (6). This would explain the slower gain in weight in the potassium and calcium mixture, the more complete subsequent recovery, and also, to some extent, the fact that muscles which have been in the mixtures lose weight more slowly when subsequently immersed in Ringer's solution than do the muscles which have been in the pure potassium solutions. We thought that more light might be thrown on this question by immersing companion muscles in 0.9 per cent potassium chloride, and then transferring one to Ringer's solution and the other to pure 0.7 per cent sodium chloride solution and comparing the rapidity with which they lost weight. If it be supposed that the surfaces of the muscle fibers are permeable to potassium chloride, but still more or less impermeable to sodium chloride after their stay in the former solution, and that calcium renders them less permeable to the potassium chloride, the muscles ought to lose weight faster in the pure sodium chloride solution than in the Ringer solution which contains calcium. The results of these experiments are curious—the muscles show a tendency to maintain in the pure sodium chloride solution the weight which they happened to have when immersed in it. See Experiments 6, 7, 8 and 9. But the results are not sufficiently uniform to warrant any particular conclusion.

The diffusion of potassium chloride into sartorii immersed in 0.9 per cent solutions of it. It is a question of great interest to what extent the potassium chloride diffuses into muscle immersed in it, and how much chlorine is left after the recovery in Ringer's solution. We have analyzed

[2] A 1 per cent calcium chloride solution has about the same calculated osmotic pressure as a 0.9 per cent potassium chloride solution.

[3] Similar results were obtained in another pair of experiments, which we have not thought it necessary to publish.

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muscle for chlorine after a stay of some hours in 0.9 per cent potassium chloride solution, and also after it has been for some hours in this solution and then recovered its irritability in Ringer's solution. See Experiments 10, 11, 12 and 13.

The results of Experiments 10 and 12 indicate that when muscle is immersed in 0.9 per cent potassium chloride solution, the salt rapidly diffuses into the fibers. In both experiments the proportion of chlorine found in the muscle after its stay in the solution was larger than could be accounted for by supposing that it takes up the solution as such. To use a somewhat crude expression, the potassium chloride diffuses into the muscle faster than the water of the solution.[4] If it be supposed that about half the original weight of the muscle consists of solids and organic water; and the other half of free water which can act as a solvent for salts (7), then it follows that at the end of Experiment 10 there was very nearly as high a concentration of potassium chloride in the total free water of the muscle as in the surrounding solution.

Experiments 11 and 13 give the chlorine content of muscles which have been for some time in 0.9 per cent potassium chloride solution, and have then recovered their irritability and returned to somewhere near their original weight in Ringer's solution. They are companions to Experiments 10 and 12. Their results show that, accompanying the loss of weight by the muscles in Ringer's solution, there is a considerable loss of chlorine. It seems difficult to interpret these results on any other supposition than that the muscle fibers, when placed in Ringer's solution after their stay in potassium chloride, are still much more permeable to potassium chloride than to sodium chloride, and that, as a consequence of this, they give up potassium chloride and water to the Ringer's solution. But perhaps the most interesting part of the result is the fact that so large a proportion of chlorine remains in the muscle after it has recovered its irritability. It is in the highest degree probable that the greater part of this chlorine left within the muscle fibers is combined with potassium. For the muscle fibers contain originally no other cation than potassium in sufficient quantity to combine with so much chlorine; and, if it were supposed that any

[4] In drawing conclusions from these experiments, the small amount of Cl found in fresh frog's muscle has been neglected. It amounts, according to Katz (Archiv für die gesammte Physiologie, 1896, lxiii, pp. 1, et seq.) to only 0.04 per cent of the weight of the muscle. It is probable that it comes chiefly from NaCl contained in the lymph spaces of the muscle, and that most of this NaCl is replaced by KCl under the conditions of Experiments 10 and 12.

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considerable part of the chlorine in question came from sodium chloride, it would follow that this had diffused in from the Ringer's solution and that the fiber surfaces were, therefore, quite permeable to sodium chloride. Under these circumstances it would be difficult or impossible to account for the loss of chlorine and water by the muscle in the Ringer solution. We have, therefore, the paradoxical result that potassium chloride within the muscle fibers is less destructive to irritability than in the interstitial spaces.

The manner in which sartorii gain weight in 0.9 per cent potassium chloride solution. Figure 1 gives two curves showing the details of the manner in which muscle gains weight in 0.9 per cent potassium chloride solution. It will be noted that in both cases the gain is more rapid somewhere between the eighth and twelfth hours than during the preceding ones. It is doubtful how this peculiarity of the curves ought to be interpreted. The idea readily occurs to one that the surfaces of the muscle fibres may become progressively more permeable to potassium chloride throughout the course of the experiment and that this change may account for the more rapid swelling in the later periods. The results of Experiments 10 and 12, however, seem to point to a different interpretation. It is to be noted that in Experiment 12, in which the muscles were immersed for only three and a half hours in the potassium chloride solution, the chlorine contained by them made up 0.92 per cent of their increase in weight. In Experiment 10, on the other hand, in which the muscles were immersed for fourteen hours and a half in the potassium chloride solution the chlorine contained by them made up only 0.69 per cent of their increase in weight. These results must be taken with some caution, as the quantities of material used for the analysis were small; but they lend themselves readily to the supposition that, when muscle is immersed in an isotonic potassium chloride solution, the salt at first diffuses in rapidly and the water comparatively slowly. Under these circumstances the water intake would become more rapid at later stages of the experiment when the osmotic pressure within the muscle fibers was raised by the presence of considerable quantities of potassium chloride.

CONCLUSION

The changes which go on in muscle immersed in a 0.9 per cent potassium chloride solution are no doubt very complicated, and we do not feel that the experiments described in the preceding pages are nearly sufficient to give a satisfactory picture of them. The following inter-

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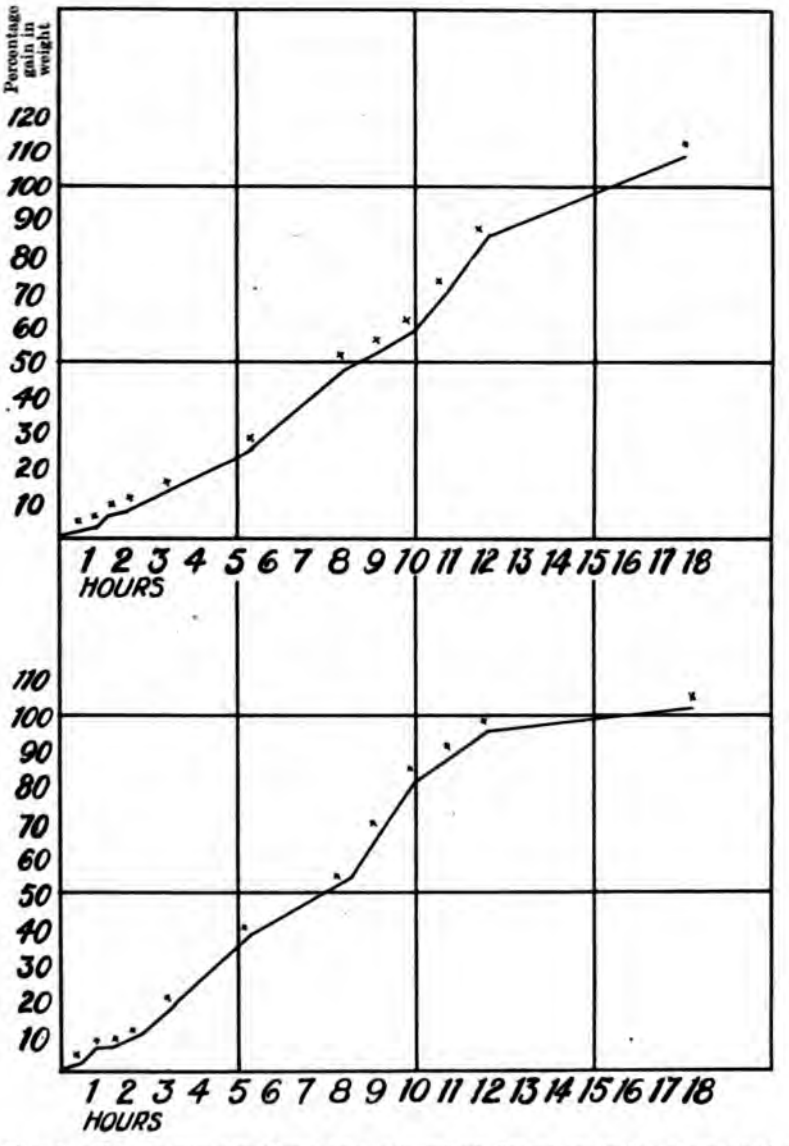


Fig. 1. Curves showing the manner in which frogs' sartorii gain weight in 0.9 per cent potassium chloride solution. Except at the periods of weighing, the muscles were kept in the ice box at between 5° and 10°.

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pretation, however, appeals to us as that which fits best the facts so far known.

The surfaces of the normal living muscle fibers are decidedly more permeable to potassium chloride than to either sodium chloride or to the potassium phosphate normally contained within them. Consequently, when a muscle is immersed in an isotonic potassium chloride solution, the salt rapidly diffuses from the solution into the fibers and raises the osmotic pressure of their contents. As a result of this the fibers take up water also from the solution. The presence of potassium chloride in the interstitial spaces of the muscle temporarily destroys its irritability, as does that of any other potassium salt.

If such an experiment be carried out at a temperature above 19 degrees, the changes described cause the muscle to produce lactic acid in considerable quantities, and this prevents it from recovering its irritability when subsequently immersed in Ringer's solution. But if the experiment be carried out at lower temperatures, the lactic acid production is small; and the muscle may recover its irritability even after it has been for twenty-four hours in the potassium chloride solution and undergone a swelling which may amount to between 50 and 100 per cent of its original weight.

A small amount of calcium chloride added to the potassium chloride solution slows the pathological changes produced by the latter and increases the period during which the muscle remains alive, just as it does when added to a sodium chloride solution. It seems not improbable that the calcium acts in both cases to preserve the normal semi-permeable properties of the surfaces of the muscle fibers.

EXPLANATION OF THE EXPERIMENTS

The experiments recorded in the succeeding protocols were all carried out on frog's sartorii, which were immersed in the various solutions, and taken out, pressed against filter paper, and weighed at the intervals indicated. The technique of the drying on filter paper and weighing was in general the same as that described by Overton (Arch. f. d. ges. Physiol., 1902, xcii, p. 126). It is to be understood that each muscle remained in each of the various solutions from the time against which the solution is first mentioned until the time against which another solution is indicated. In Experiment I, for instance, the sartorius was immersed in Ringer's solution at 11.15 a.m. July 2, and remained in that solution until 2.25 p.m. At 2.25 p.m. it was found to weigh 0.223

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gram, and immersed in 0.9 per cent KCl solution, where it remained until 8.30 p.m., when it was replaced in Ringer's solution. All the solutions were made up according to the method of Raoult; a "0.9 per cent KCl solution" means a solution made by adding 0.9 gram KCl to 100 cc. of distilled water; an "0.88 per cent KCl + 0.025 per cent $CaCl_2$ solution" means a solution made by adding 0.88 gram KCl and 0.025 gram $CaCl_2$ to 100 cc. of distilled water. The Ringer's solution used in Experiments 1 to 9 inclusive was made by adding 0.65 gram NaCl, 0.02 gram KCl, 0.025 gram $CaCl_2$, and 0.02 gram $NaHCO_3$ to 100 cc. distilled water. The Ringer's solution used in Experiments 10 to 13 inclusive was the same except that the $NaHCO_3$ was omitted. Our general experience, in addition to some preliminary experiments, which we have not thought it worth while to record in detail, lead us to believe that the survival of muscle under ordinary conditions and its recovery after treatment with KCl are litle affected by the presence or absence of $NaHCO_3$ in the Ringer solution used.

The weights of the muscles are given in grams, and, for convenience, the percentage changes in weight are given in the next column. The percentage changes are all calculated from the weight which each of the muscles had at the end of its first immersion in Ringer's solution.

During the intervals between weighings the solutions containing the muscles were kept in the ice box at temperatures which varied, as the protocols indicate, between 5 and 10°. In most cases the freshly removed muscles were placed immediately in the cold Ringer's solution. In Experiments 1, 2 and 3, as the protocols indicate, the freshly removed muscles were placed in Ringer's solution at 19°. The beakers containing the solution and the preparations were, however, immediately put in the ice box, and must have fallen to a temperature below 10° in the course of a few minutes.

In regard to the conventions used to indicate the degree of irritability of the preparations, it need only be said that "Irritability+" means that the muscle gave only a small contraction when stimulated by a strong current; "Irritability+++" means normal irritability; and "Irritability++," an intermediate degree.

In cases where one experiment is said to be a "control" or a "companion" for another, it is meant that the two experiments were carried out on the same day on opposite sartorii from the same frog.

Experiments 10 to 13 inclusive need no particular comment except that the chlorine estimations were made gravimetrically. As a control for our method of chlorine analysis we analyzed 2.073 grams of fresh

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frog's muscle and found 0.058 per cent Cl. The figure given for the chlorine content of fresh frog's muscle by Katz (Arch. f. d. ges. Physiol., 1896, lxiii, 1 et seq.) is 0.0402 per cent.

PROTOCOLS OF THE EXPERIMENTS

Experiment 1. July 2 to 5, 1915

TIME	SOLUTION	WEIGHT	PERCENTAGE CHANGE IN WEIGHT	TEMPERATURE	REMARKS
July 2			*per cent*	°C.	
11.15 a.m.	Ringer			19	
2.25 p.m.....	0.9 per cent KCl	0.223		7	
8.30 p.m.....	Ringer	0.258	15.7	7	
July 3					
9.30 a.m.....	Ringer	0.221	−0.9	7	Irritability+++
July 4					
9.00 a.m.....	Ringer	0.215	−3.6	9	Irritability+++
July 5					
8.45 a.m.....	Ringer	0.211	−5.4	7	Irritability+++

Experiment 2. July 2 to 5, 1915

July 2					
11.30 a.m.....	Ringer			19	
2.15 p.m.....	0.9 per cent KCl	0.231		7	
July 3					
7.30 a.m.....	Ringer	0.355	53.7	7	
12.07 p.m.....	Ringer	0.310	34.3	9	Unirritable
2.15 p.m.....	Ringer			9	Irritability+
July 4					
9.30 a.m.....	Ringer	0.261	13	9	Irritability++
July 5					
9.00 a.m.....	Ringer			7	Irritability++

Experiment 3. July 2 to 5, 1915

July 2					
11.45 a.m.....	Ringer			19	
2.45 p.m.....	0.9 per cent KCl	0.215		7	
July 3					
2.45 p.m.....	Ringer	0.430	100	9	
July 4					
9.00 a.m.....	Ringer	0.275	28	9	Irritability+
July 5					
9.00 a.m.....	Ringer			7	Irritability+

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Experiment 4. July 14 and 15, 1915

TIME	SOLUTION	WEIGHT	PERCENTAGE CHANGE IN WEIGHT	TEMPERATURE	REMARKS
July 14			*per cent*	°C.	
9.25 a.m.....	Ringer			8	
11.25 a.m.....	0.88 per cent KCl + 0.025 per cent $CaCl_2$	0.114		8	
3.25 p.m.....	0.88 per cent KCl + 0.025 per cent $CaCl_2$	0.147	28.9	7	
5.25 p.m.....	Ringer	0.157	37.7	7	
7.25 p.m.....	Ringer	0.142	24.6	7	
9.25 p.m.....	Ringer	0.125	9.6	7	
July 15					
9.25 a.m.....	Ringer	0.127	11.4	7	Irritability+++

Experiment 5. Control for Experiment 4, July 14 and 15, 1915

July 14					
9.30 a.m.....	Ringer			8	
11.30 a.m.....	0.9 per cent KCl	0.107		8	
3.30 p.m.....	0.9 per cent KCl	0.149	39.3	7	
5.30 p.m.....	Ringer	0.163	52.3	7	
7.30 p.m.....	Ringer	0.144	34.6	7	
9.30 p.m.....	Ringer	0.127	18.7	7	
July 15					
9.30 a.m.....	Ringer	0.130	21.5	7	Irritability++

Experiment 6. July 15 and 16, 1915

July 15					
2.35 p.m.....	Ringer			7	
July 16					
10.05 a.m.....	Ringer	0.114		8	
11.05 a.m.....	0.9 per cent KCl	0.110		8	
2.05 p.m.....	0.7 per cent NaCl	0.125	13.6	6	
2.35 p.m.....	0.7 per cent NaCl	0.126	14.5	7	
3.35 p.m.....	0.7 per cent NaCl	0.126	14.5	7	
5.30 p.m.....	0.7 per cent NaCl	0.126	14.5	7	Irritability++

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Experiment 7. Control for Experiment 6, July 15 and 16, 1915

TIME	SOLUTION	WEIGHT	PERCENTAGE CHANGE IN WEIGHT	TEMPERATURE	REMARKS
July 15			*per cent*	*°C.*	
2.30 p.m.....	Ringer			7	
July 16					
10 a.m.....	Ringer	0.109		8	
11 a.m.....	0.9 per cent KCl	0.114		8	
2 p.m.....	Ringer	0.135	18.4	6	
2.30 p.m.....	Ringer	0.136	19.3	7	
3.30 p.m.....	Ringer	0.132	15.8	7	
5.30 p.m.....	Ringer	0.130	14.0	7	Irritability+++

Experiment 8. July 15 and 16, 1915

July 15					
2.45 p.m.....	Ringer			7	
July 16					
10.15 a.m.....	Ringer	0.99		8	
11.15 a.m.....	0.9 per cent KCl	0.102		8	
2.15 p.m.....	0.7 per cent NaCl	0.110	7.8	6	
2.45 p.m.....	0.7 per cent NaCl	0.107	4.9	7	
3.45 p.m.....	0.7 per cent NaCl	0.107	4.9	7	
5.45 p.m.....	0.7 per cent NaCl	0.109	6.9	7	Irritability++

Experiment 9. Control for Experiment 8, July 15 and 16, 1915

July 15					
2.40 p.m.....	Ringer			7	
July 16					
10.10 a.m.....	Ringer	0.098		8	
11.10 a.m.....	0.9 per cent KCl	0.094		8	
2.10 p.m.....	Ringer	0.107	13.8	6	
2.40 p.m.....	Ringer	0.108	14.9	7	
3.40 p.m.....	Ringer	0.102	8.5	7	
5.40 p.m.....	Ringer	0.101	7.4	7	Irritability+++

Experiment 10. July 21 and 22, 1915

10.35 to 11.30 a.m. Sartorii from 5 frogs (one from each frog) dissected out and placed in Ringer's solution at 8 to 9°.

11.30 p.m., July 21 to 12.15 a.m. July 22. These muscles found to weigh 0.857 gram; transferred to 0.9 per cent KCl solution.

2.15 to 3.05 p.m., July 22. These muscles weighed 1.520 grams. They were fused with Na_2O_2 and analyzed for Cl. They yielded 0.0188 gram AgCl, equivalent to 0.0046 gram Cl, or to 0.0097 gram KCl.

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Experiment 11. Companion to Experiment 10, July 21 to 23, 1915

10.35 to 11.30 a.m. The other sartorii from the frogs used in Experiment 10 were dissected out and placed in Ringer's solution at 8 to 9°.

11.30 p.m., July 21, to 12.15 a.m., July 22. These muscles were found to weigh 0.871 gram; they were transferred to 0.9 per cent KCl solution.

2.15 to 3.05 p.m., July 22. These muscles weighed 1.610 grams; transferred to Ringer's solution.

9.30 to 9.50 a.m., July 23. These muscles weighed 0.985 gram; all found to be moderately irritable. They were fused with Na_2O_2 and yielded 0.0107 gram AgCl, equivalent to 0.0026 gram Cl, or to 0.0055 gram KCl.

Experiment 12. September 8, 1915

11.37 a.m. to 12.15 p.m. Sartorii from 6 frogs (one from each frog) removed and placed in Ringer's solution at 8 to 9°.

4.45 to 5.40 p.m. These muscles found to weigh 1.054 grams; transferred to 0.9 per cent KCl solution.

8.15 to 9.10 p.m. These muscles weighed 1.337 grams. They were fused with Na_2O_2, and yielded 0.0106 gram AgCl, equivalent to 0.0026 gram Cl, or to 0.0055 gram KCl.

Experiment 13. Companion to Experiment 12, September 8 and 9, 1915

11.37 a.m. to 12.15 p.m., September 8. The other sartorii from the frogs used in Experiment 12 were dissected out and placed in Ringer's solution at 8 to 9°.

4.45 to 5.40 p.m. These muscles found to weigh 1.067 grams; transferred to 0.9 per cent KCl solution.

8.15 to 9.10 p.m. These muscles weighed 1.344 grams; transferred to Ringer's solution.

10.00 to 10.34 a.m., September 9. These muscles weighed 1.140 grams. One was only slightly irritable; all the rest were moderately irritable. They were fused with Na_2O_2 and yielded 0.0088 gram AgCl, equivalent to 0.0022 gram Cl, or to 0.0047 gram KCl.

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THE INFLUENCE OF AGE UPON THE VENOUS BLOOD PRESSURE IN MAN

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In the course of observations directed to other ends it was noted that the venous blood pressure was appreciably lower in boys than in men of mature years. Accordingly, when opportunity offered in the summer of 1915, a series of observations on men of different age groups were collected and are reported in this paper.

The method used has been described elsewhere (1). Because of the uncertainties of illumination the pressures were read at complete collapse of the vein and not, as is more accurate, at the point at which the shadow comes and goes with slight oscillations of the outside pressure. This tends to give somewhat higher values but under the present circumstances the comparative value of the pressures is undoubtedly more reliable. In collecting the data all obviously abnormal cases, particularly in regard to cardiac lesions, were excluded. The children were found in orphan asylums and in schools; the boys in schools and shops; the young men in colleges and shops; the men in shops and the old men in institutions for the aged. Several of the groups were completed with ambulatory surgical cases in the dispensary and hospital. In all cases the observations were made in the trunk-vertical position.

The accompanying figure (fig. 1) presents the data obtained. The venous pressure readings, expressed in centimeters of water and referred to the heart level, are plotted on the abscissae and the ages of the subjects on the ordinates. The average of all the readings in the several decades in which not less than fifty observations were made are presented in the form of a curve. These average figures are as follows:

YEARS	CM.	YEARS	CM.
5–15	8.30	45–55	19.04
15–25	12.66	55–65	24.17
25–35	15.00	65–75	25.59
35–45	17.98	75–85	26.00

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These values show clearly that there is a continuous rise in venous pressure as age progresses. If, however, averages are made for the half-decades in which not less than twenty-five observations were made, the rise of pressure is less regular as the following figures show.

YEARS	VENOUS PRESSURE	INCREASE IN PRESSURE OVER PRECEDING HALF DECADE
	cm.	
5–10	6.36	—
10–15	9.97	3.61
15–20	11.58	1.61
20–25	13.39	1.81
25–30	13.88	0.49
30–35	16.08	2.20
35–40	17.22	1.14
40–45	18.80	0.58
45–50	17.85	−0.95
50–55	20.28	2.43
55–60	24.03	3.75
60–65	24.29	0.26
65–70	24.27	−0.02
70–75	26.44	2.17
75–80	26.11	−0.33
80–85	25.88	−0.23

A curve plotted from these figures shows irregularities between 25 and 30, 45 and 50, and 60 and 70 years. I am not inclined to stress these irregularities because it is not improbable that they would be smoothed out if the number of observations was increased. It will be seen that the maximum pressure occurs between 70 and 75 years and that the averages for the two half-decades following are both lower. Whether there is any significance in this drop of venous pressure toward the end of life may be doubted but the fact that it appears to prevail over a number of years suggests that it may be indicative of the failure of some compensatory mechanism.

Clark (2) found in a study of cardiac cases that a venous pressure persisting and rising above 20 cm. foretold the approach of clinical signs of decompensation and that such a condition, in patients confined to bed, usually indicated a fatal outcome. His cases ranged around 50 years of age for which age the normal average pressure, as here given, is 19.04 cm. It is inconceivable that the healthy individuals in my series should exhibit a venous pressure so close to the danger zone as

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noted by Clark. Clark's criterion for reading the pressure was the point at which the shadow of the vein comes and goes with slight oscillations of the outside pressure which is more accurate and gives lower values than the criterion which I was obliged to employ, namely, the

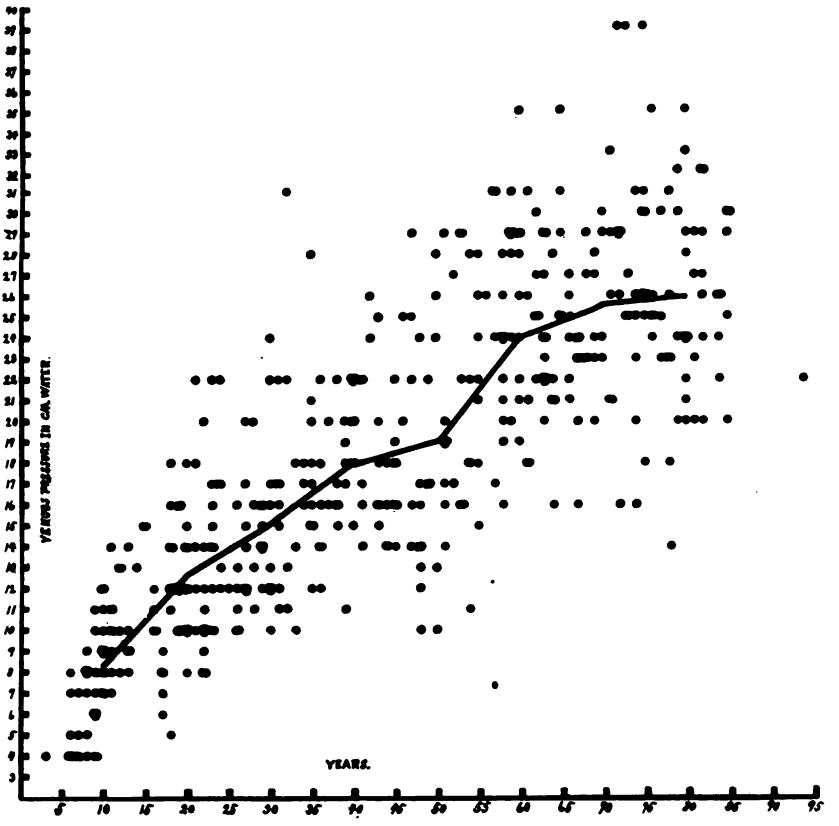


Fig. 1. Venous blood pressure observations on men. To show the rise in venous pressure which occurs as age progresses. The dots represent individual observations. The curve represents the average of not less than fifty observations in each of the several decades.

point of complete collapse of the vein. This difference in method might give values differing a couple centimeters. Furthermore, my subjects were up and about and active, Clark's cases were confined to bed in a semi-recumbent position. It is not known what effect protracted quiet has on the venous pressure but it is probably safe to assume that it

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would result in lowered values. Finally, Clark's cases presumably had some myocardial weakness, the result of which might well be that the heart was unable to function under a feeding pressure that in normal individuals causes no embarrassment whatsoever. It would seem, therefore, that the results here reported are not necessarily in conflict with Clark's conclusion that a pressure of 20 cm. in cardiac cases is dangerous.

The figure shows profound variations in the venous pressure in all periods of life. Doubtless every healthy individual exhibits a normal variation attributable in part to the condition of bodily activity and to the diurnal rhythm (1). In the series of observations here reported the first of these could not well be controlled and no correction for the second was made. That there are still other factors contributing to normal variations in venous pressure may be assumed but they have not been investigated. Allowing, however, for these normal variations, it is probable that still other causes must be invoked to account for the extreme differences found in each age period. Probably the ages given by the subjects are in the main reliable except in the case of the very old in whom there appeared to be a tendency to in-accurate statement. The resistance of the skin and the rigidity of the venous wall would obviously influence the amount of pressure required to collapse the vein but the few cases exhibiting such conditions were excluded. The most probable cause of these extreme variations would seem to lie in the condition of bodily health, the relation of which to venous pressure, except in cardiovascular cases, is as yet unknown. Effort was made, as has been stated, to exclude from the series all but healthy individuals, but in a large series poorly under control and in which only the grossest condition of general health was established it seems not improbable that numerous abnormal conditions might go undetected which would be capable of causing at least some of the extreme variations in pressure observed.

The factor or factors contributing to this age-rise of venous pressure are unknown. Casual inspection of animals suggests that the pressure may vary directly with the size or weight of the body. If we assume that man has reached maturity at 40 years and thereafter does not gain in size or weight (3) we may be justified in considering the first part of the present curve of venous pressure as an expression of growth. The curve of growth shows a fairly constant rise until 17 years is reached and thereafter flattens, at first slowly then more rapidly until 40 years. In a general way the curve of venous pressure exhibits a similar trend.

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After this period the curve of venous pressure is less regular although the rise persists. The suggestion that this later rise represents a functional compensation for the weakened heart muscle of age is attractive but too many other changes in the cardio-vascular mechanism are involved to warrant any but the most qualified consideration of the suggestion.

It is interesting to note that young men in exercise may raise their venous pressure to the height found in old men at rest as the following figures show:

AGE	BEFORE EXERCISE	AFTER EXERCISE
	cm.	*cm.*
26	8	26
19	12	24
20	15	24
20	10	24
39	11	28
29	—	32

The first value given was obtained before and during exercise on a stationary bicycle; the others were obtained before and some minutes after a competitive five-mile run. While these figures doubtless do not represent the maximum venous pressure which the human heart can sustain they bear a striking relationship to the values found in old age, a relationship which suggests a common causative factor.

Finally, a possibility to account for the progressive rise in venous pressure in the later years of life is a change in position of the right auricle with respect to the subcostal angle. One gets the impression on palpating for the subcostal angle that it lies lower on the trunk in old people. This may be due to a bony rigidity which defines the point of palpation more sharply and at a lower level than in the young. If such a condition exists it would lead to higher pressure readings. But it is generally stated that the thorax assumes a more conical form as years pass and it is also stated that the base of the heart is lower in old people than in younger ones (4). If these statements are accepted the pressure readings would tend to be lower, so it would seem that any change in the assumed position of the heart, if one exists, in people of different ages must be ascribed to differences in rigidity of the thorax. Such rigidity and the expansion of the lower part of the thorax would

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increase the prominence of the tip of the sternum, thereby giving a lower point of palpation. Such differences cannot, however, be great enough to account for all of the rise in venous pressure noted in old people.

CONCLUSIONS

Observations on the venous blood pressure in men of different ages are presented which show a progressive rise in venous pressure practically throughout life.

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THE CHIEF PHYSICAL MECHANISMS CONCERNED IN CLINICAL METHODS OF MEASURING BLOOD PRESSURE[1]

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This paper attempts to analyze the chief physical mechanisms concerned in clinical methods of measuring systolic and diastolic blood pressure with the use of an arm band or plethysmograph, where the arm band is inflated to a high pressure until the radial pulse is suppressed, and then is gradually lowered until the radial pulse reappears. This is taken as the systolic pressure. Or else graphic tracings are taken of the pressure in the arm band, so that when the pressure is high in the arm band no oscillations in the tracing are produced by the heart beat. But as the pressure in the arm band is gradually lowered a point is reached where arterial pulsations are feebly transmitted through the cuff pressure and appear in the tracing. This point was formerly taken by many as the systolic pressure. At a somewhat lower level the tracing often shows a more rapid augmentation of the arterial pulsatory oscillations and often it also shows at about this point a widening of the limbs and also somewhere near this point there is often noted a notch in the downward limb. The various points are taken by others to be the systolic pressure. From these levels on down, as the arm band pressure is gradually lowered, the arterial pulse waves recorded on the tracing are augmented until they reach the maximum. Some point on this maximal oscillation, or else the first diminished beat is the most commonly selected criterion for obtaining the diastolic pressure. How-

[1] A preliminary report of this work was made in 1914 at the St. Louis meeting of the American Physiological Society. Also some of the results were presented in a paper read before the Allegheny County Medical Society, February 16, 1915, a brief abstract of which was published in the Bulletin of that society. The later results were presented before the 1915 meeting of the American Physiological Society at Boston.

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ever, all do not agree. As the arm band pressure is still further lowered, the tracings of arterial pulsation gradually diminish and finally disappear. Another series of criteria for locating the systolic and diastolic blood pressures are certain of the various phases of sounds produced and heard through a stethoscope when the arm band is compressing the artery at different levels of pressure.

The results of the present study lead to the conclusion that none of these various above described commonly accepted criteria for obtaining the systolic and diastolic blood pressures yield correct measurements, but give readings which are too high. The amount of error depends upon and varies directly as the resistance of the arterial wall to compression and distension.

This means, among other things, that the results show that the greatest pulsatory oscillations obtained from a plethysmographic tracing of a finger or limb, or from an arm band are not obtained when the counterpoise pressure is exactly equal to the diastolic pressure, in other words, that the commonly accepted principle of Marey (1) does not hold. Marey argued that in this state the blood vessels would be completely relaxed so that their walls would fluctuate freely as if floating passively between the pressures of the blood within the vessel and that of the counterpoise pressure without the vessel. But we hold that Marey's principle is not true even provided the oscillations of blood pressure be infinitely small, or that the movement of mercury in the manometer be infinitely small or that the dilation of the vessel during systole be infinitely small. For, we argue, the moment the blood pressure begins to rise above the diastolic, at once the blood vessel must begin to be distended. This distention must be sufficient to allow the displacement necessary to fill the space in the mercury manometer caused by the elevated pressure. Therefore, necessarily, the instant the blood pressure begins to rise above the diastolic, there will be interference with the transmission of the arterial pulse wave, or the transmitted oscillation will be damped by the stretched blood vessel. As a matter of fact, the most favorable point for the transmission of extremely minute arterial oscillations of pressure is where the vessel is in a partially collapsed condition, for here is possible the easiest fluctuation in volume with the least bending of the walls of the vessel. The sides of the vessel in this condition are not flattened but are curved (fig. 8, *B*). So Marey's principle is not true even under theoretical conditions.

It is upon the Marey principle that the most commonly accepted

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clinical methods of measuring diastolic blood pressure rest, for as mentioned above, the practice is to take as the criterion of diastolic pressure the lowest point on the greatest oscillation or the first beat where the amplitude of the oscillations begins to decline. Likewise, until very recently critical scientific opinion has been almost as overwhelming in favor of the Marey principle. However, some have not accepted it. For example, Martin (2) advocated raising external pressure till diminution occurred, and then lowering it till diminution occurred and that the average be taken as the mean blood pressure. Also, Mosso (3) thought he measured the mean pressure by taking the mean point on the greatest oscillation (instead of the lowest point). Roy and Adami (4) claimed the true diastolic pressure was slightly (4 to 5 mm. of mercury) below that of the point of greatest oscillations. Hill and Bernard (5) took the lowest point of the greatest oscillation to be the mean blood pressure. Oliver (6) held that the greatest oscillations occur at the mean blood pressure. The excellent work of MacWilliam and Melvin (7) was not seen by us until our work was about half completed. They found that the greatest oscillations occurred at a point where the counterpoise pressure was raised sufficiently above the true diastolic to cause the blood vessels to be completely flattened at the diastolic level. But when using a larger rubber tube it was observed to be only half flattened at the diastolic level. They did not explain why this was so. Our results offer an explanation for the apparent discrepancy. Wiggers (8) calls attention to interesting work by Christen (9). However, we have been unable to obtain access to the original publication.

PLAN OF EXPERIMENTATION

The plan of experimentation adopted was to use the simplest possible physical models to work out the underlying physical principles involved in the process of making clinical blood pressure measurements with the arm band or plethysmograph. Complicated models with artificial heart, aorta, other vessels, peripheral resistance and artificial heart beat of normal rate and circulatory fluid in motion, were thought to make difficult the recognition and analysis of the processes going on. The pressure was controlled by raising and lowering pressure bottles. These changes in pressure which were graphically recorded were made accurately and slowly enough to permit the easy analysis of the results.

The sounds produced were studied and the mechanism of their production ascertained as completely as possible at this time.

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After the physical experiments were fairly well advanced a series of experiments on the dog were performed according to the following plan: The dog's own heart beat was stopped but the pressure curve in the aorta was maintained artificially by a supply of warm 0.9 per cent NaCl mixed with blood. It was so arranged that pulsations were made from a certain known and controlled minimum or diastolic pressure up to a certain known and controlled maximum or systolic pressure. The pulsations were made slowly enough to permit easy analysis of the tracings. In this way the pressure curve in the aorta was a known quantity. Readings by the arm band method were then made and compared with the actual pressure.

The results of the physical experiments were found to be applicable to and in agreement with those of the animal experiments.

EXPERIMENTAL RESULTS

A good many physical experiments of a very elementary character were performed which are not included in this paper for the reason that they are already well known and they do not bear directly on the problem under investigation here. Even some that are reported here are not offered as new or original; but they are given in order to form a foundation for the explanation and illustration of what takes place when the blood pressure is measured by the arm band method.

The volume pressure curve of a rubber membrane was tested. A thin piece of rubber tissue was stretched lightly over the mouth of a thistle tube which was inserted upward through the bottom of a two-holed rubber stopper. A glass tube was inserted through the other hole in the rubber stopper. The stopper was inserted into a wide mouthed bottle. The outer end of the thistle tube was then connected with a pressure bottle which also communicated with a mercury manometer which traced on a kymograph. The other glass tube was fitted with a narrow nozzle tip and bent down to discharge into the top of a burette.

The zero point was obtained by filling the whole system with just the amount of water so that the tip of the discharging nozzle just dipped under water in a beaker, while the level of the top of the water in the pressure bottle was on a level with that of the water in the beaker, and with the rubber diaphragm completely relaxed and the pressure equalized on each side of it. Then the beaker of water was removed and at the same time the pressure bottle was very slightly lowered to the level of the opening of the nozzle. The curve of volume as a func-

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tion of pressure was then taken either by successively raising the bottle a certain distance, or else by successively raising it gradually until a certain volume of water had been discharged into the burette (fig. 1).

The results of these experiments are shown in the curve in fig. 2. In studying the curve it is seen that at first when pressure is brought on the membrane it gives way easily and a relatively slight change in pressure causes a large fluctuation in volume. This results in a bulging of the membrane which is gradually stretched assuming a more and

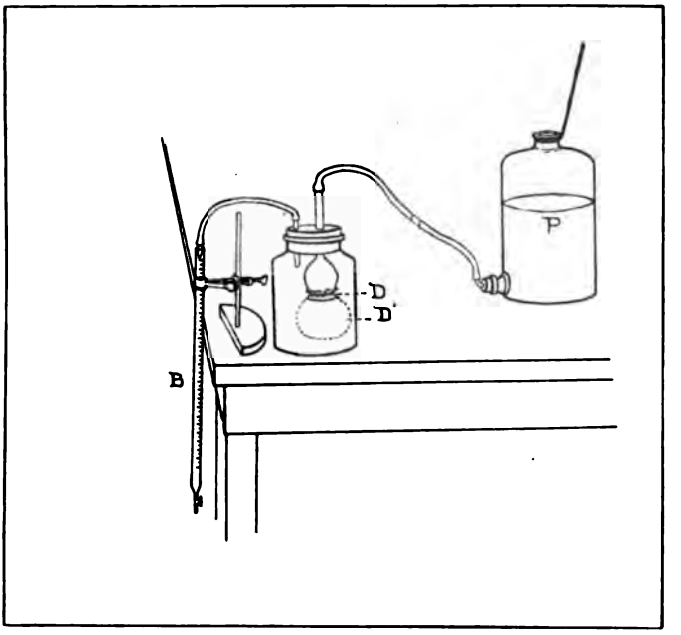


Fig. 1. Arrangement of apparatus for obtaining volume pressure curve of rubber diaphragm. *P*, pressure bottle; *B*, burette; *D*, rubber diaphragm relaxed; *D'*, rubber diaphragm distended.

more convex shape. As this occurs there is a diminution of the rate of volume change as compared with the rate of pressure change. So the volume-pressure curve bends upward. After a time, when the membrane is so far bulged outward that it assumes a more rounded shape, the relation alters so that the volume increases more rapidly than the pressure does. Still later, when the walls of the membrane have become so thinned that they form a large sphere attached at one side to the thistle tube, the pressure does not need to increase any further

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in order to produce a further increase in volume. In short, the now large rubber balloon would burst if kept under that constant pressure. It has reached the blow-out phase. In this state, it is like the urinary bladder and other physiological spherical sacs, which when they are

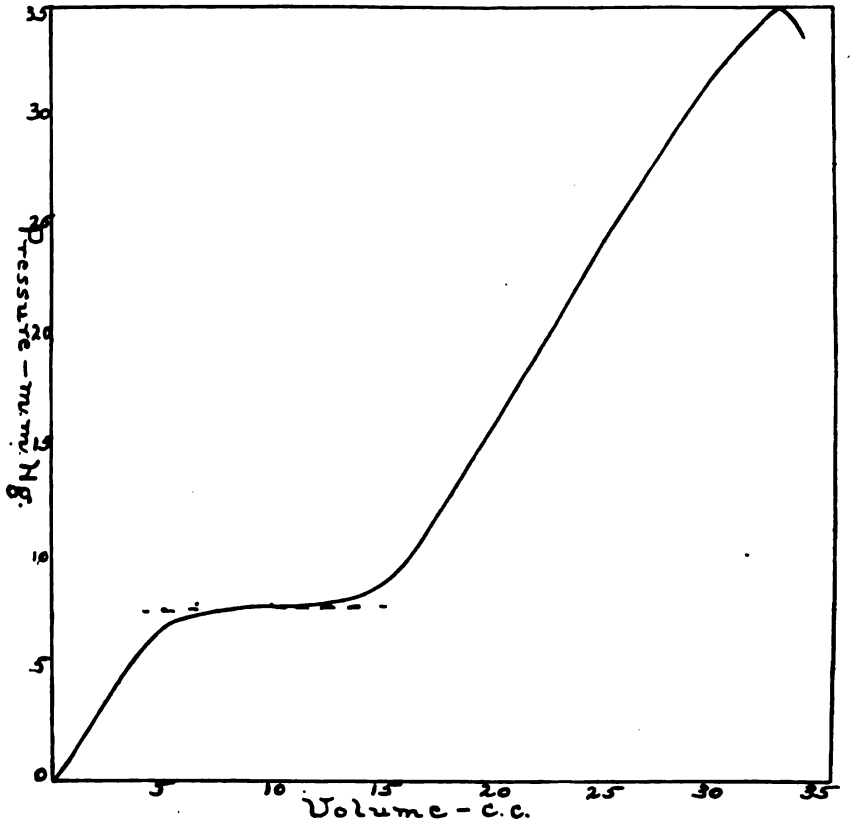


Fig. 2. Curve of volume changes of rubber diaphragm under various pressures. - - - - - Level where inside and outside pressures are equal.

well filled, but not over distended, have no higher pressure than when partially filled.

This line of experimentation prepares us for the results given below that when we seek to measure a curve of pulsatory pressure on the one side of a membrane by taking a tracing of the oscillations transmitted by this pressure to the space on the other side of the membrane we get

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the following result: By using the same apparatus as in figure 1, but connecting a recording manometer in place of the narrow tipped nozzle and connecting also a pressure bottle in this system, oscillations of pressure can be made on one side of the membrane which are transmitted to the other side. One side we elect as representing the inside or arterial pressure, the other will be the outside or arm band pressure (fig. 3).

Now, after having placed the inside pressure bottle at diastolic level, if the outside pressure bottle is raised to a high point, the mem-

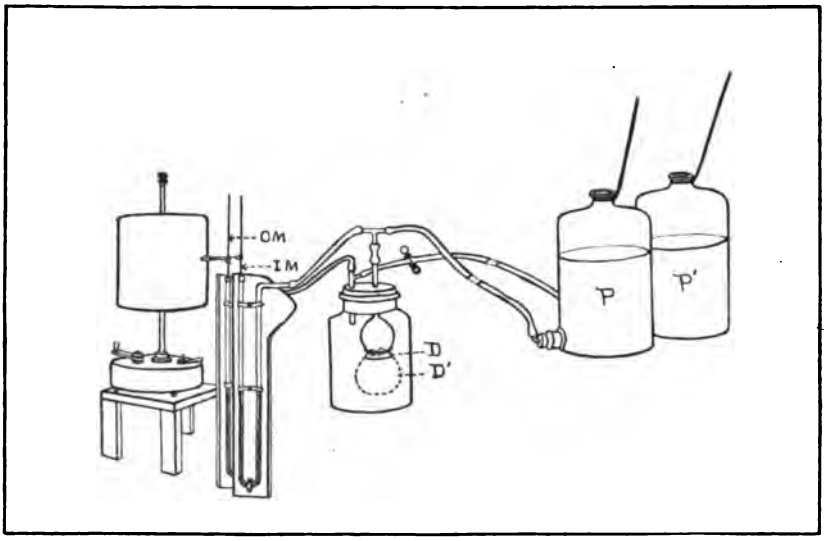


Fig. 3. Arrangement of apparatus for recording height of oscillations made on one side of rubber diaphragm and the resulting transmitted oscillations. *P*, inside pressure bottle; *P'*, outside pressure bottle; *IM*, inside manometer; *OM*, outside manometer; *D*, rubber diaphragm collapsed; *D'*, rubber diaphragm distended.

brane bulges in and adapts itself to the inside of the thistle tube. In this state, if the inside pressure bottle is raised from the diastolic to the systolic level, there will be only a slight wave of the pulsatory pressure transmitted to the outside manometer for the stretched membrane applied against the inside of the thistle tube prevents it. Now the outside pressure is gradually lowered and the transmitted pulse oscillations increase until they reach a maximum. This maximum is reached at the point where the *mean* of the *inside* pulsatory pressure

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is just equal to the *mean outside* pressure. In short, the membrane offers less resistance to the pulse wave when the inside pressure is just equal to the outside pressure. And in order to reduce the damping effect of the membrane to the lowest limit, any given pulse oscillation must swing equally above and below this favorable point (fig. 4, *A*). If we start with the *diastolic point* of *inside* pressure just equal to the *diastolic point* of *outside pressure*, (which ought to produce maximal oscillations according to Marey's principle), the oscillations transmitted and recorded on the tracing by the outside manometer are not so great as they were in the above experiment. The reason for this is that the membrane is at a favorable point of complete relaxation at the beginning of the pulse wave, but it must become more and more stretched as the pressure rises above the diastolic level (fig. 4, *B*). The reverse is true when the *inside pressure* at *systolic* level is just balanced by the

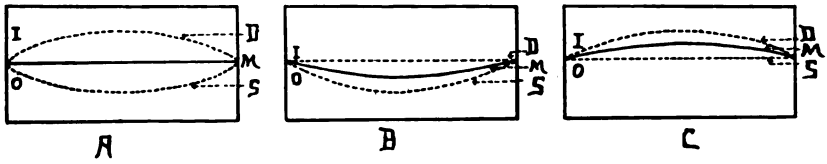


Fig. 4. Diagram showing movements of rubber diaphragm caused by oscillations in pressure. *I*, inside pressure space; *O*, outside pressure space; *A*, when mean inside and outside pressures are equal; *B*, when diastolic inside and outside pressures are equal; *C*, when systolic inside and outside pressures are equal; *D*, *M*, *S*, position of rubber diaphragm at diastolic, mean and systolic pressures.

systolic point of *outside* pressure. Here again, the transmitted oscillations are not so great as those where the mean inside pressure is just equal to the outside pressure (fig. 4, *C*).

Therefore, where the *mean inside pressure* is just equal to the *mean outside* pressure is the *most favorable point* for the transmission of pulsatory waves through a rubber diaphragm, and therefore, it is the point of greatest transmitted pulsatory oscillations.[2]

Although the above principle is true for a rubber membrane which is flat and which under high pressure bulges out and becomes spherical in shape, it does not follow that it must be true for a rubber tube or for blood vessels, which are cylindrical in shape.

The curve of volume pressure of a rubber tube of a thinness and a soft-

[2] This is true within the lower limits of the elasticity of the diaphragm. If the inside diastolic pressure is so great as to distend the diaphragm into a spherical balloon with thin walls, this law is altered. But that need not be discussed in this paper.

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ness rather similar to that of a large blood vessel was taken in the same manner as was the curve for the diaphragm described above. The curve is different from that of the rubber diaphragm. That is, the volume-pressure curve of the rubber tube, within the limits of pressure used, passes through three phases (fig. 5).[3] The first phase is where

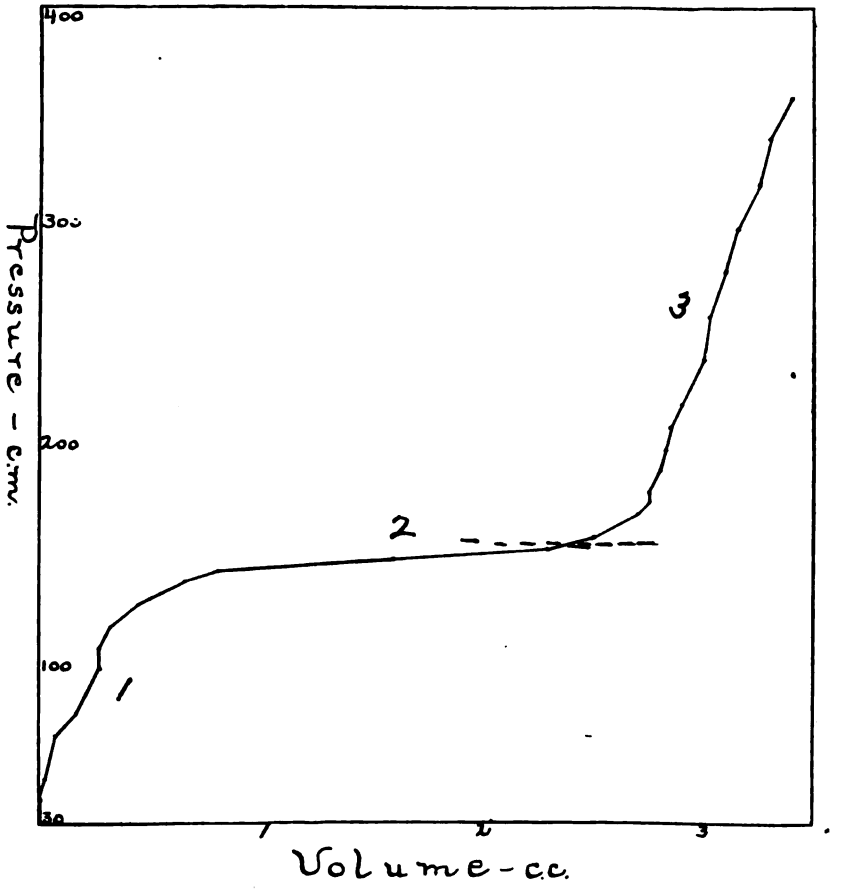


Fig. 5. Curve of volume change of rubber tube under various pressures. *1*, first phase; *2*, second phase; *3*, third phase;- - - - level where inside and outside pressures are equal.

[3] If the inside pressure were increased to the bursting point of the tube the curve would be altered as was the case in the rubber balloon phase of the diaphragm experiment, but this need not be entered into here, because it is not encountered in taking blood pressure readings.

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the outside pressure is sufficiently higher than the inside to cause the vessel to be collapsed. Here a given increase in pressure inside the tube gives the least change in volume because the vessel is more or less completely collapsed and the transmission of pressure is damped by the outside pressure which holds the tube more or less tightly shut. In the second phase there is the greatest change in volume with a given change in pressure because the vessel merely changes in shape from the collapsed, flat closed to the passively rounded, open. This does not require stretching of the walls of the vessel, but only requires bending of them. In the third phase the tube is open and round. An increase in volume requires increase in circumference of the tube. This requires that the walls of the rubber tube be actually stretched; not merely bent. So here, as in the first phase, the increase in volume is small with a given increase in pressure; but it is not as small as in the first phase.

Other conditions being equal, the smaller the calibre of the tube, the greater are the differences between the three phases of the curves.

Next in order is the consideration of the transmission of pulsatory oscillations made by the inside pressure and recorded by the outside pressure. The arrangement of the apparatus is shown in figure 6. It is similar in principle to that described above for the rubber diaphragm experiment.

Beginning with the outside pressure at so high a point that the rubber tube was flatly collapsed thereby, oscillations of the inside pressure were made slowly and accurately by raising and lowering the inside pressure bottle. A level was arbitrarily selected as the diastolic pressure, and another level conveniently located above it was arbitrarily taken for the systolic pressure. In this way the pulse oscillations throughout the experiment were controlled and kept at the same level. They were made slowly and steadily so that the tracing would be accurate.

The results of this series of experiments are as follows: Beginning with the outside pressure at quite a high level and making pulsatory oscillations in the inside pressure from the diastolic to the systolic level it was found that very tiny oscillations were transmitted to the outside manometer. It was very difficult to raise the outside pressure to such a great height that no oscillations whatever were transmitted. In other words, the point where the oscillations first appeared was far above the true systolic pressure. *The height above the systolic at which*

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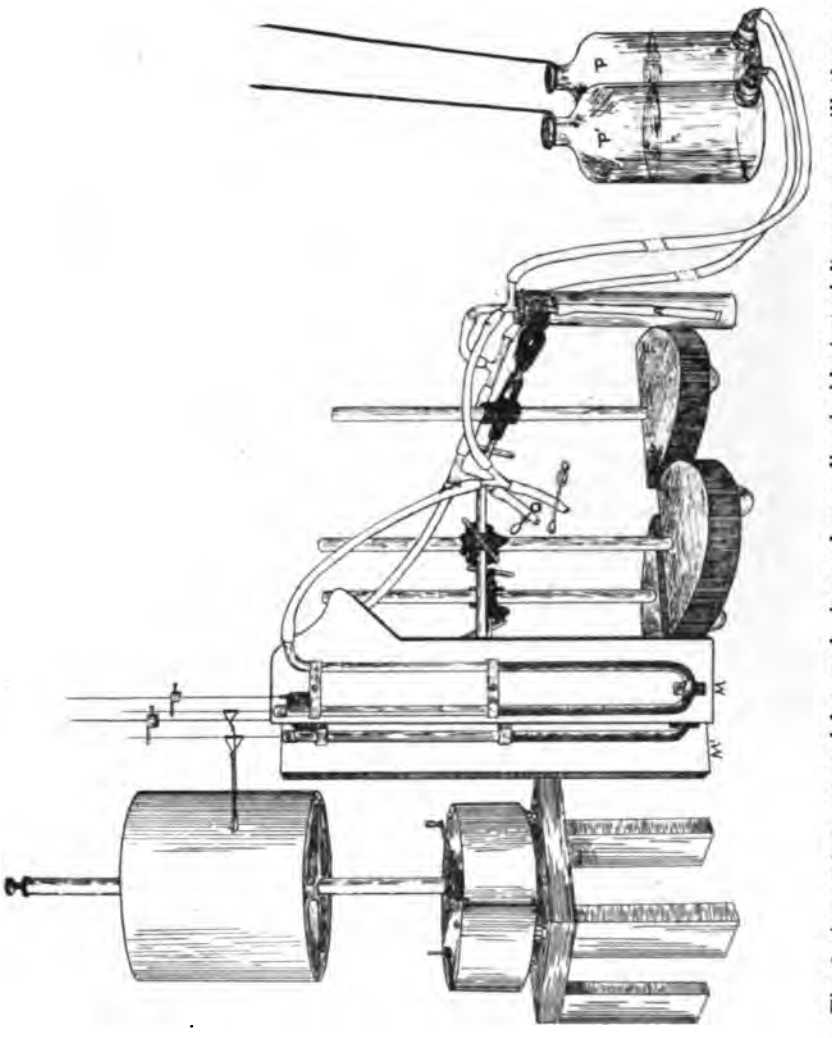


Fig. 6. Apparatus arranged for producing and recording inside (arterial) pressure oscillations and the resulting transmitted outside (arm band) pulsatory oscillations. *A*, artery or rubber tube; *P*, arterial or inside pressure bottle; *P'*, arm band or outside pressure bottle; *M*, arterial or inside manometer; *M'*, arm band or outside manometer.

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they first appeared varied directly as the delicacy of the tracing mechanism, and also directly as the resistance of the vessel.

Then, as the pressure in the outside system, or as the outside pressure bottle was lowered, the transmitted pulsations were correspondingly gradually augmented until they reached a maximal. *This point of maximal pulsations however, was at a much higher level than the true diastolic pressure.*

As the outside pressure bottle was further lowered there was adecrease in the transmitted oscillations until they became very small.

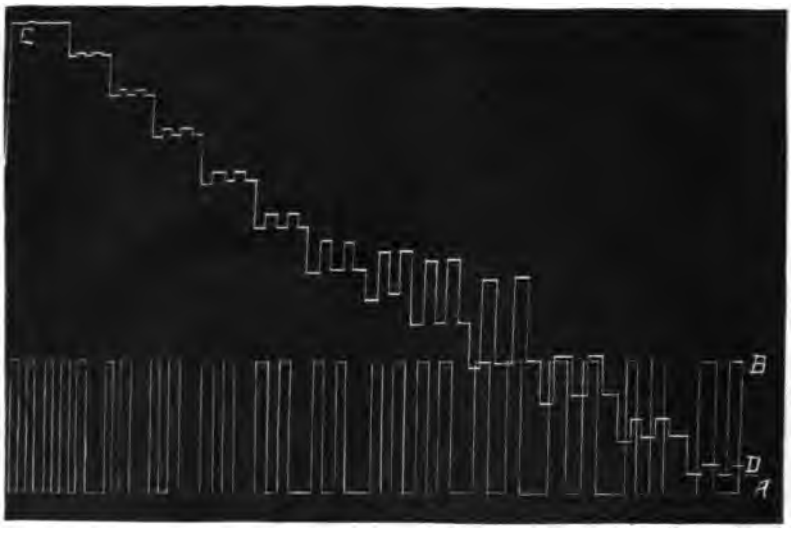


Fig. 7. Tracing showing transmitted pulsatory oscillations made by inside pressure and recorded by outside pressure. *A–B*, actual oscillations inside of rubber tube; *C–D*, transmitted oscillations.

And, like those small oscillations seen when the outside pressure was extremely high, it was very difficult to reach a point where they disappeared completely, and also like them, the point to which they descended varied directly as the delicacy of the tracing mechanism.

Figure 7 shows a typical tracing taken as just described. Note that in this instance, even the lowest point of the greatest oscillations is at a point not only above the diastolic, but even above the systolic pressure.

The alterations in the shape of the vessel or the rubber tube during the taking of this tracing are interesting and significant. In the ordi-

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nary experiments with our model, at the highest point on the tracing where the outside pressure is high above the inside pressure, the tube is completely collapsed, except when there are two little spaces, one at each corner of the flattened tube, which are not completely closed (fig. 8, *A*). These unclosed spaces at each corner open slightly when the inside pressure rises from diastolic up to systolic level. As the outside pressure is lowered these spaces increase in size until they attain a size where the arterial tube opens very slightly when the inside pressure reaches systolic level (fig. 8, *B*). As the outside pressure is still further lowered, the arterial tube continues to open wider until it reaches the point where the greatest transmitted oscillations are traced. At this level, at the diastolic pressure the arterial tube is more or less completely collapsed, at the mean pressure the tube has become about half collapsed and at systolic pressure it has rounded and filled out and then become slightly stretched (fig. 8, *C*). The degree of fluctuation depends

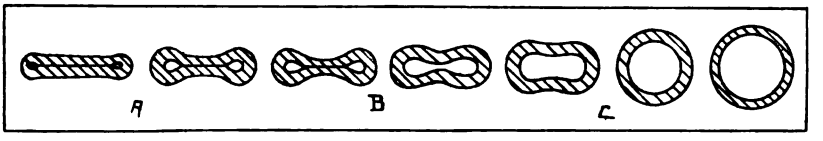


Fig. 8. Cross-section of vessel or rubber tube showing changes in shape during oscillations of pressure. *A*, when outside pressure is high above inside pressure, tube completely or almost completely collapsed. *B*, outside pressure lower than *A*, vessel partly open; *C*, outside pressure still further lowered.

upon the ratio of volume of the arterial tube to the volume of the manometer.

Here, as with the diaphragm experiment, there is a favorable point where oscillations are most readily transmitted. It is not at the point where mean inside pressure exactly equals mean outside pressure, because the curve of volume-pressure of the rubber tube is not the same above this point as it is below it. In this regard it does not behave like the rubber membrane. For within limits it is more difficult to expand the rubber tube more completely above the favorable point than it is to collapse it more completely. Therefore, in order to compensate for this inequality in the tube's curve of pressure, the greatest oscillations may be obtained if, when beginning the tracing, the mean of the outside pressure be elevated above the mean of the arterial or inside pressure. Just how much of this compensatory elevation is necessary depends upon (1) the pressure-volume curve of that partic-

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ular arterial tube; (2) upon the amplitude of the inside pulsatory oscillations; and (3) upon the volume of displacement necessary in the outside mercury manometer in order to accommodate itself to the change of pressure.

That is (1) the larger the calibre and the thinner and softer the walls of the tube, the more the curve of volume pressure would be extended, and therefore, a slight change in pressure would cause a great increase in volume, or in other words, there would be only slight damping of a transmitted pulse wave, in which case the "compensatory elevation" required would be slight. But, on the other hand, the thicker and more rigid the tube and the smaller its calibre, the greater would be the necessary compensatory elevation.

Also, (2) when the inside pulsatory oscillations are small the "compensatory elevation" is slight, but the greater the inside oscillations the greater must be the "compensatory elevation" of outside pressure.

And (3) if the calibre of the outside manometer is small, only a slight change in volume is required to fill up the space displaced in the proximal limb of the mercury which is depressed by the increase in pressure. Therefore, the arterial tube need not fluctuate greatly in volume in order to transmit the required pressure wave. In this case, the "compensatory elevation" of outside pressure would be relatively small. But when the outside manometer is large in calibre it will require large volume fluctuation of the arterial tube. This in turn will necessitate a large compensatory elevation.

So the favorable point above and below which the oscillations swing is not a definitely fixed point, but varies with the conditions of the experiment. However, *it is always necessary to have the mean outside pressure more or less elevated above the mean inside pressure.*

There is here, as in the diaphragm experiment described above, a "most favorable point" at which oscillations can be transmitted with the least damping. But it is unlike the diaphragm experiment in this regard: In the diaphragm experiment this most favorable level is a fixed point which never alters with altered amplitude of arterial pulsations, nor with thickness of the rubber diaphragm, nor with the calibre of the mercury manometer; whereas with the rubber tube experiment, this most favorable point is a movable point. That is, the point changes with the amplitude of arterial pulsations, with the ratio of the volume of the rubber tube to the calibre of the manometer. The reason for this difference between the location of the favorable point in the diaphragm and in the elastic tube is found in the volume-pressure

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curves of these two mechanisms. The diaphragm transmits inside oscillations with least damping when the *mean* inside pressure is just equal to the *mean* outside pressure, for in this state the diaphragm is most completely relaxed and can fluctuate most freely inward or outward by bending and without much stretching. The essential point is that *the volume-pressure curves above and below this point are identical.* That is, the increased damping is equally increased whether the inside pulsation is negative or positive, or in other words whether it extends above or below this point. Therefore, no matter how great the inside pulsation, it must swing equally above and below this favorable point in order to suffer the least amount of damping. So also it is with the factor of calibre of the manometer. The greater the calibre of the manometer the greater must be the volume displaced by the movement of the column of mercury in a given change of pressure recorded by the manometer. Therefore, the greater the manometer's calibre the greater will be the amount of fluctuation of the diaphragm to displace the required amount of mercury. This means that the greater the calibre of the manometer the greater the damping effect caused by the stretching of the membrane. Nevertheless this greater damping effect would be exercised equally and identically above and below the most favorable point. Therefore, changes in the calibre of the manometer do not change the position of this most favorable point for transmitting oscillations.

Consider now, the same factors in the elastic tube experiment. With the elastic tube it was found that the most favorable level is not a fixed point, but varied with the conditions of the experiment. It varied with the amplitude of the arterial pulsations, with the calibre of the manometer, and with the calibre of the tube, and with the stiffness of the tube. The essential point here is that the volume-pressure curve is *not identical* above and below the point where the mean arterial pressure is just equal to the mean transmitted pressure, nor is it identical above and below any other point. But as seen above, the three phases of elasticity of the rubber tube included in this study are all different from one another. Therefore, the most favorable area on this curve for the transmission of any given inside oscillation will vary according to the amount of fluctuation of the tube which is caused by the oscillation. If the amount of fluctuation is extremely small the area is more toward the top of the second phase (fig. 9). As the arterial pulsations increase in magnitude, requiring greater fluctuation of the tube, the favorable region on the curve includes that part of the curve

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which was used for the small pulsation, but there is added to it a lesser part from above and a greater part from below, when there is included all of the region in the upper and middle portion of the second phase. As arterial pulsations continue to increase in magnitude, the whole of the second phase is included together with a small portion of the third phase. After this, continued increase in magnitude of pulsations causes the addition of larger amounts of the third phase, with lesser amount of the second phase.

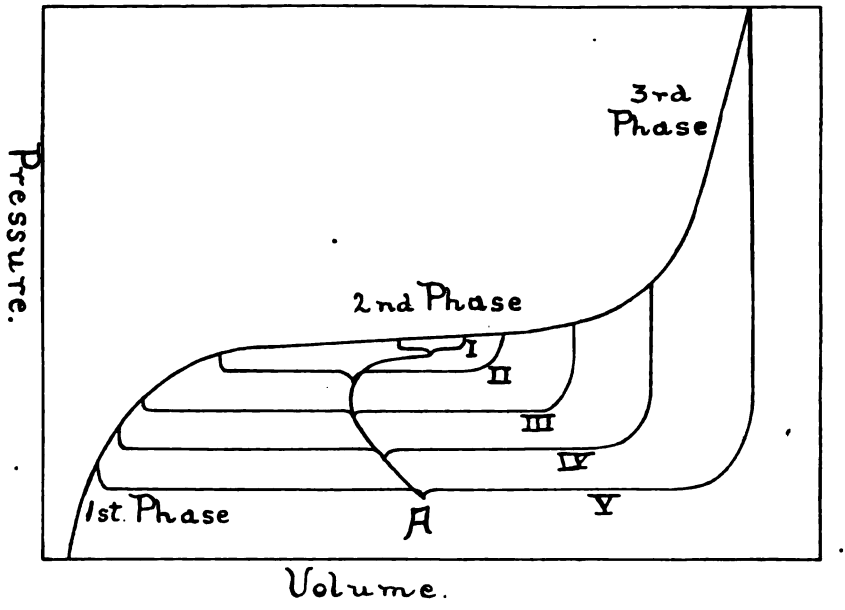


Fig. 9. I, II, III, IV, V, area of volume-pressure curve occupied by maximal transmitted oscillations when arterial oscillations are of different magnitude. I, minute arterial oscillation; *V*, large arterial oscillation; *A*, Alteration of mean point on maximal transmitted oscillation.

Thus, with very minute arterial pulsations, the *mean point* of the maximal transmitted oscillation is at the point where the outside pressure is at a level above the point where *mean* arterial is just equal to *mean* transmitted oscillation. As arterial oscillations are increased in amplitude, the *mean point* of transmitted oscillations moves upward. That is, the outside pressure must be increased in order to obtain maximal oscillations. This upward movement of the mean of outside maximal pulsation continues until the arterial pulse has increased to

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such amplitude that the vessel is carried through the whole of the second phase and the lower portion of the third phase of the volume-pressure curve of the arterial tube. After this, further increase in amplitude of arterial pulsation causes the mean point of maximal outside pulsation to move downward again, for the third phase of the elastic tube transmits more favorably than does the first phase, and the increased fluctuations of the tube are made more in the third phase, and less in the first phase.

Or, in other words, as the arterial pulse wave increases from the very small pulse, on up to a large pulse wave, at first the mean outside pressure must be raised sufficiently above the mean inside pressure to just partially collapse the vessel. In this state the least amount of bending of its walls will give the required fluctuation in volume. This is the most favorable point for transmission of these minute oscillations. As the arterial pulsations are increased in amplitude, the mean outside pressure must be raised higher than it was with the small arterial pulsations, because the vessel is more easily bent inward or collapsed than it is bent outward or expanded, as is shown by the volume-pressure curve in this region. That is, when sufficient extra outside pressure is made the tube partially collapses because the outside pressure overcomes the natural tendency of the tube to assume a rounded shape. In this partially collapsed state the outside pressure balances the elasticity outward pull of the vessel toward the rounded shape. Here it is easier to swing the walls inward and further collapse the tube than it is to swing them outward and further dilate the tube. Therefore, as the arterial pulsations are increased in amplitude from the small oscillations up to somewhat larger ones, the outside pressure must be raised in order to secure maximal transmitted oscillations, for in so doing the advantage is taken of using more of the second phase where the fluctuation is easier, and using less of the upper and part of the second phase where fluctuation is more difficult (fig. 9). As the arterial pulsations are further increased, the mean outside pressure must continue to be raised still further in order to continue to make use of a greater added portion of the easy inward bend of the collapsing tube, and of a lesser added portion of the difficult fluctuation of the outward expanding tube.

This process continues until at the diastolic arterial pressure the vessel is so far collapsed that its walls are touching in the middle (fig. 8, *B*), or it has reached the top of the first phase of the volume-pressure curve where oscillations are transmitted with more difficulty than they are in the third phase (figs. 5, 9).

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Therefore, from now on, as the arterial pulsations are further augmented it is easier to provide the increased fluctuation of the volume of the arterial tube by extra expansion with extra stretching of the walls during systole, than by extra compression with extra flattening of the already almost completely collapsed vessel. This requires that the mean of maximal transmitted oscillation shall turn about and instead of moving upward shall move downward, or shall not move upward, or at least shall not move upward as fast as before, i.e., upward or downward as regards the height of the cuff or outside pressure. So much for the effect of alterations in amplitude of arterial pulse on the location of the mean point of maximal transmitted oscillations. A similar discussion could be carried through either as regards calibre of manometer; resistance of vessel; or calibre of vessel. However, since the philosophy is very much the same in all these factors, it is deemed unnecessary to go through it here.

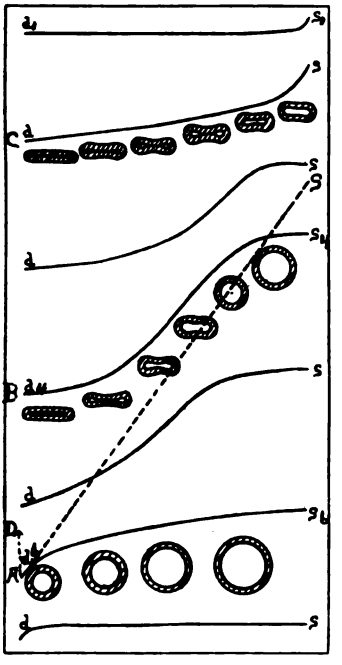


Fig. 10. Diagram showing curve of arterial or inside pressure from diastolic up to systolic and the resulting transmitted pressure curves produced when arm band or outside pressure is placed at various levels, together with the accompanying changes in the shape of the vessel. *D–S*, diastolic to systolic—true pressure; *d–s*, curves of transmitted oscillations from diastolic to systolic pressures, when outside pressure is placed at various levels.

Further light is cast upon the essential points of the whole problem by tracing with the above described apparatus on a moving drum, curves showing, not only the amplitude of the inside and outside pressure changes, but the form of the curves made by such a tracing. In short, while the drum was moving at a constant speed the inside pressure was raised from the diastolic point to the systolic point at a constant speed. This caused the inside pressure manometer to trace a straight line which was directed upward and to the right at an angle of about forty-five degrees with the base line (fig. 10, *D*, *S*.) Then the inside pressure bottle was lowered, at the same constant speed, back to diastolic level. These tracings were several

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times repeated, the inside manometer traveling over the same line, but starting with the outside pressure at different levels.

It is noted that when beginning with the outside pressure high (fig. 10, d_1, s_1), there is very little change in the height of the outside pressure which was almost a straight line. When beginning with the outside pressure somewhat lower, there is very little change in the outside pressure curve, as the inside pressure rises from the diastolic up to the systolic level, until at the very last just before reaching systolic, when there is a sharp upward hook to the curve. It is also noted here that the arterial tube is rather tightly collapsed until the point where this sharp upward hook occurs, and there the tube begins to open slightly. Beginning with the outside pressure set at lower levels, the outside pressure curve alters as shown in the tracing and diagram in figure 10, d_4, s_4. Here where the point of greatest transmitted oscillations is reached it is noted that, beginning at the diastolic point, the outside pressure curve is at first slow in rising; then it rises sharply, and then more slowly again. It traces an S-shaped three phase curve, which is similar to the volume-pressure curve of the rubber tube. Also, it is noted that the arterial tube is more or less completely flattened at the beginning where the curve is slow in rising, then the tube opens out and becomes round in the region where the abrupt rise in pressure is noted, and the tube is partly distended in the upper region where the curve is again slowed down.

When beginning with the outside pressure low, say with inside and outside pressure equalized at the diastolic point (the point where according to Marey the greatest transmitted oscillations ought to occur (fig. 10, d_6, s_6) the transmitted oscillations are small. At first the arterial tube is passively rounded and the curve starts upward, but as the inside pressure continues to rise the arterial tube becomes partly distended at which point the curve sharply bends over and does not rise much afterward, while the inside pressure is continuing on its way up to systolic level.

These experiments are identical with those just described above except they show a graphic curve of the whole course and shape of the pressure curves on a moving drum, instead of merely showing their amplitude on a stationary drum. They show that the most favorable region for the transmission of pressure oscillations is when the arterial tube's walls are partially collapsed and can therefore fluctuate inward and outward, changing the volume easily, without involving any actual stretching of the walls. This required that the mean outside pressure must be higher than the mean inside pressure.

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With these principles in mind a series of animal experiments was performed, as was mentioned in the introduction. The object of these animal experiments was to find out where the first oscillations occur and also where the greatest oscillations occur when an arm band was placed about a dog's neck, instead of a patient's arm, and when the pressure in the cuff was raised to a high point and then gradually lowered. The arrangement is shown in figure 11. But in order to

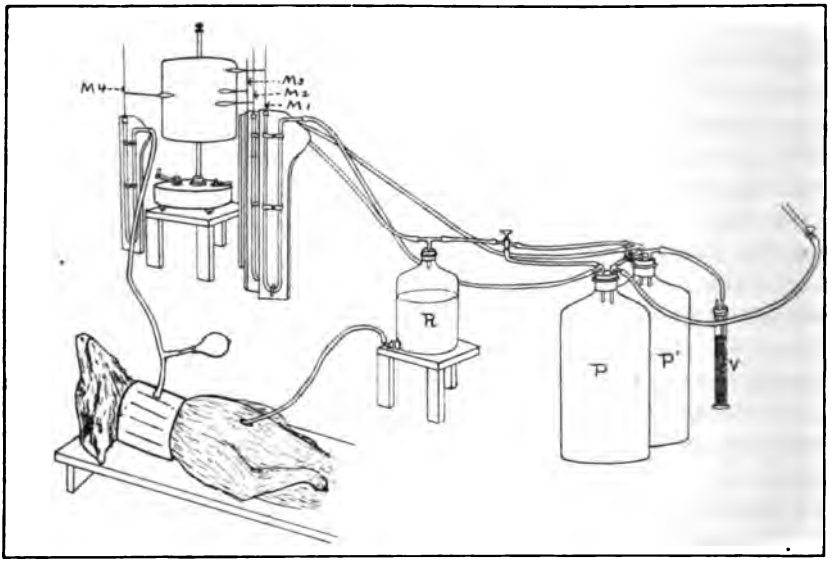


Fig. 11. Arrangement of apparatus for producing artificial pulsatory oscillations of pressure in dog's aorta and for recording the oscillations transmitted by an arm band placed about the dog's neck. *P*, systolic pressure; *P'*, diastolic pressure; *R*, reservoir of defibrinated blood; *M1*, systolic pressure manometer; *M2*, diastolic pressure manometer; *M3*, manometer recording artificial pulsatory oscillations; *M4*, manometer recording arm band pressure transmitted oscillations; *V*, mercury valve.

attain this object, an artificial heart beat was substituted for the dog's own heart beat. The purpose of this was to have a known diastolic and systolic pressure in the aorta and great vessels and to make the pulse beats slow enough to permit the mercury manometer to register accurately. For, when experiments in this field are attempted using the dog's own heart beat, the actual pressure curve in the aorta is not known. Maximal and minimal valves do not remedy this difficulty for they select and record only the highest and lowest beats and there-

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by they do not measure the general course of the average diastolic nor that of the average systolic pressure and furthermore they are not accurate, whereas the oscillations of the manometer that are used to measure the diastolic and the systolic pressures are not oscillations produced only by the highest and lowest beats but they are produced by a series of uniform beats. Furthermore, if the rate of the heart beat coincides with the period of the manometer the oscillations are greatly augmented, but when the rate of pulse beat is such that it clashes with the period of the manometer the oscillations of the manometer are greatly diminished. So that a given pulsatory pressure, when its rhythm is coincident with the period of the manometer, may cause oscillations of the manometer which are several times greater than the same pulsatory pressure at a rhythm which interferes or does not coincide with the period of the manometer.

Therefore, to eliminate the unknown and variable quantity of the dog's own heart beat, an artificial pulse beat was used; and to eliminate the error of augmentation of oscillation of the manometer caused by coincidence or the error of diminution of oscillations caused by interference of rhythm with the period of the manometer, the artificial pulsations were made so slowly that neither interference not coincidence could occur.

The dog was anesthetized with ether and a snugly fitting cannula was inserted into the abdominal aorta close under the diaphragm. By a large rubber tube the cannula was connected with a large three-way cock which led to two large reservoir bottles one on each side, containing blood mixed with 0.9 per cent NaCl. One of these bottles was kept at high pressure (systolic), and the other at a low pressure (diastolic). Each bottle had connected with it a manometer which traced on the drum throughout the experiment. The heads of pressure in these two bottles were kept constant, as was shown by their respective manometers, by having the high pressure or systolic bottle freely connected with the air pressure system of the laboratory, which is a forty gallon tank with a safety valve and the pressure supplied by an air pump run by an electric motor. While the experiment was in progress the safety valve was set at the desired (systolic) pressure and the pump was kept going so that some air was constantly blowing off. This maintained the systolic pressure bottle at a constant. Leading from the air pressure system was another connection which led to the low (diastolic) pressure bottle; but this was a narrow connection. That is, the rubber tube was almost closed by a screw clip, so that a small stream

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of air from the air pressure system was constantly blowing through the narrow slit into the low pressure reservoir. In order to hold the pressure in this bottle at the desired (diastolic) level, a mercury valve (or a glass tube dipping under mercury) was connected with the bottle. So by adjusting the depth of the tube under the mercury the pressure of the air in the bottle could be controlled. When the valve was so adjusted and the pressures had become equalized there was always a small stream of air bubbling through the mercury valve.

When all was ready the dog's trachea was opened and a long metal tracheal cannula inserted far down the trachea, or else if a short cannula was used the trachea was dissected out and turned upward. Then an arm band was placed about the dog's neck which had been shaved. The arm band included the cannula if it could be done without shutting off the dog's respirations; if not, the trachea and tracheal cannula were not included within the arm band. The arm band pressure was traced by a manometer on a drum. Now the dog's heart was stopped by crowding the anesthetic. Next the artificial arterial pulsations were made by turning the three-way cock now right now left, connecting the aorta first with the systolic then with the diastolic pressure bottles.



Fig. 12. Blood pressure tracing, arm band around dog's neck, cannula in abdominal aorta, arterial pulsations of pressure made artificially.

The cuff pressure was raised to a high point and allowed to fall gradually. The tracings showed a great similarity to those described above on the artificial model. The main points to be noted are similar to those noted above on the experiments on the model. The first oscillations begin at a point above the true maximal or systolic pressure, the height being directly proportional to the delicacy of the technique. The greatest oscillations on the tracing are at a point above the true diastolic pressure (fig. 12).

In most of our experiments this error is 1 or 2 cm. Since the animals were normal and their blood vessels therefore soft and not sclerotic and since they were deeply under the anesthetic, this small error ought not to be surprising. If we had encountered a rigid pair of carotids we doubtless would have found a much greater error.

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As regards the mechanism of the production of the sounds which are used for criteria in measuring blood pressure by auscultation, the work of MacWilliam and Melvin (7) is for the most part correct. However, we have approached the problem somewhat differently, and have some additional observations to report.

An artificial model was prepared with a soft piece of rubber tubing of suitable length placed inside a glass tube and connected by a three-way cock with a high or systolic pressure bottle, and also with a low

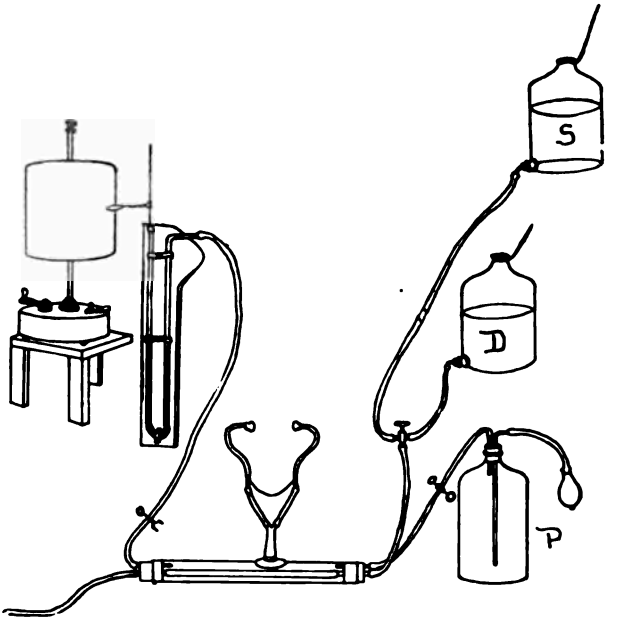


Fig. 13. Arrangement for studying the mechanism of the production of sounds used in auditory blood pressure method.

or diastolic pressure bottle. The bottles were placed at the desired levels. Pulsations were made inside the rubber tube by turning the glass three-way cock first on the systolic and then on the diastolic pressure bottle. The piece of rubber tube connected through short brass tubes through rubber corks to the sources of pressure on one end and to a short soft piece of rubber tubing on the other end (fig. 13). Through another brass tube inserted through the rubber cork, air pressure was made in the space outside the rubber tube and within the large glass tube. This outside space, through a T-tube communicated with the

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mercury manometer. Also in some experiments, a venous return rubber tube was brought back through the outside air space of the large glass tube and in some no peripheral tube at all was used. A stethoscope was sealed on to the side of the glass tube with wax or paste. When this was done, sounds were produced which roughly correspond with those produced in the artery which is compressed by the arm band during the determination of blood pressure by the auditory method.

The results were as follows:

The first sharp sound (first phase) which is heard when the arm band pressure is gradually lowered, occurs when the elastic vessel or tube is tightly closed except at the top of systole at which point a small spurt of fluid escapes through the narrow slit between the sides of the collapsed vessel.

The first dull sound (second phase) which is heard when the arm band or outside pressure is still further lowered, occurs when it is quickly opened and closed at each pulse beat.

The second sharp sound (third phase) occurs when the vessel is open except at the bottom of diastole, at which point the vessel closes for an instant and in doing so reduces the column of fluid to a small jet which passes through the narrow silt between the collapsed vessel walls.

The second short dull sound (fourth phase) occurs when the vessel is at no time completely closed, not even at diastole, but the vessel is partly collapsed at diastole and fairly well, but not completely distended at systole.

These facts regarding the relation of the vessel to the production of the sounds are presented without at present trying to account decisively for all the various other conditions which are associated with the production of these sounds.

However, it would seem that the nozzle-swish of the fluid through the narrow orifice may well be the chief factor in the production of this sharp sound of the first and third phases.

Also, for the second phase, the sudden opening and closing of the vessel together with the sudden joining and separation of the column of liquid may well be the chief factors in the production of these sounds.

In the fourth phase the stretching and partial collapsing of the vessel, together with its disturbing effects on the flow of liquid may well be the factors concerned in the mechanism of the production of these sounds.

The relation and importance of these and other various conditions we hope to study in the future.

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SUMMARY AND CONCLUSIONS

The commonly accepted criteria for obtaining the systolic and diastolic blood pressures do not yield correct results, but give readings which are too high. The amount of error depends upon and varies directly as the resistance of the vessels to compression and expansion.

That is, the true systolic pressure is somewhat lower than the point where the arm band pressure is just sufficient to cut off the radial pulse, and it is also lower than the point where a manometer, connected with the arm band pressure, when the arm band pressure is gradually lowered from a high point, shows the beginning of oscillations. The height at which these beginning oscillations first appear depends upon the delicacy of the recording mechanism. The true systolic pressure may also be lower than the various points in the tracing of arm band pressure where there are certain alterations in the form of the tracing; a dicrotic notch, widening of the limbs, a sudden increase of augmentation. The exact significance of these latter criteria is not yet known.

That is, secondly, the true diastolic pressure is somewhere lower than the lowest point of the greatest oscillations that are produced by the manometer connected with the arm band pressure, when the arm band pressure is varied until the point of greatest oscillations is located.

This is contrary to Marey's principle.

In order to employ these criteria to measure blood pressure a correction is necessary. Unfortunately the amount of correction varies with the resistance of the arteries to compression and expansion. In soft arteries the error is not large, but in arteries made resistant by disease or by contraction of their muscular elements, it must be very great.

This same error is inherent in the auditory as well as the graphic method of measuring blood pressure.

The sharp sounds of the first and third phases of the auditory method of measuring blood pressure occur when the blood is passing through a narrow slit between the almost completely collapsed vessel. Therefore, the sounds may be due to a nozzle-swish action.

The first dull sound heard in the second phase occurs when the vessel opens and closes quickly. Therefore, the sounds may be due to the stretching of the walls, the sudden closure of the vessel, or the alteration in the flow of blood through the vessel, sudden separation and joining of the column of blood.

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The second dull sound heard in the fourth phase occurs when the vessel, even during diastole, is not completely closed, but it is partly collapsed in diastole and partly distended during systole. Therefore, the sound may be due to the partial collapsing and the partial distention of the vessel together with the disturbing effects of this on the blood flow through the vessel.

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SUPPLEMENTARY NOTE

We are surprised to find just as we go to press that Joseph Erlanger has adopted the main idea of our work which we presented and demonstrated in his laboratory at the December, 1914, meeting of the American Physiological Society, and has published it in this JOURNAL in the number just prior to the present number. We are pleased thus to have a good portion of our work confirmed just before its appearance in the JOURNAL, but we regret that our long deliberation has resulted in the confirming paper appearing just before the original contribution.

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STUDIES ON ABSORPTION FROM SEROUS CAVITIES

I. The Omentum as a Factor in Absorption from the Peritoneal Cavity

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The studies which have been made from time to time on the mechanism of absorption of granules from the peritoneal cavity and the pathways over which such absorption takes place have all tended to emphasize the importance of the diaphragm as the active agent in this process, and have indicated the diaphragmatic lymphatics as the channels through which granular material of all sorts passes to reach the lymph glands and other organs in which it is eventually found. Although pathologists and clinicians have long been aware of the activity of the omentum in peritonitides of various sorts, the possibility of an absorptive function on the part of the omentum has been either discredited or disregarded. Indeed there is noticeable a decided disposition on the part of the various investigators who have studied the problems which the drainage of the peritoneal cavity presents, to evade discussing the question of the omentum's share in ridding the peritoneal cavity of foreign substances, a disposition which is excusable in the light of the difficulties which obtaining a definite answer to the question presents. For example MacCallum (1) ignores the omentum entirely. Buxton and Torrey (2), discussing the possibility of an exodus of granular material from the peritoneal cavity by other channels than the diaphragmatic lymphatics speak of absorption via the omentum in the following words:

> The omentum appears to furnish such an additional channel to some extent. Sections made from the omentum within an hour after injection of lamp black into the peritoneal cavity show that the afferent plexuses of the lymph nodes are filled with free particles. After two or three hours the particles have been taken up by the macrophages and lie principally in the sinuses of the node.
>
> All this corresponds precisely with processes seen in the anterior mediastinal lymph nodes, so without going into further details we may take it as probable

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that the lymphatics of the omentum, as well as those of the diaphragm, are concerned in the initial rapid rush of particles to the organs, though probably to a less extent.

They believe that the mass of the foreign material which can be found in the rolled up omentum after the induction of a foreign body peritonitis is merely adherent to or buried in the tissue of that organ.

Those authors who admit the possibilities of the omentum as an absorptive pathway are unanimous in the belief that absorption of granules takes place via the omental lymphatics. In the absence of definite knowledge of the facts of the case such a conclusion as regards the absorption of particulate matter is logical when we consider the part which lymphatic radicles play in the removal of granules from any locality, and the ease with which granular material injected into the peritoneal cavity may be recovered from the lymphatic glands. However, it is by no means certain that there are any lymphatics in the omentum at all. Ranvier (3) has shown that while lymphatic vessels may be found in new born kittens they are obliterated and disappear before the animal has reached adult age. If they are present they are certainly not numerous or easy to demonstrate, the silver technique which brings out their outline so beautifully in the diaphragm and other regions failing to show them in this situation.

The absorption of true solutions from the peritoneal cavity has been the subject of extensive researches, which have for their object the establishment of the blood stream or the lymphatic channels as the pathway by which fluids leave the peritoneum. Melzer and Adler (4) have studied these absorption routes by injecting solutions of strychnine and potassium ferrocyanide into the peritonea of normal animals in which the entrance of lymph into the blood stream had been prevented by previous ligation of both innominate veins. They found that strychnine convulsions and the appearance of the Prussian blue reaction in the urine were much delayed in animals whose innominate veins had been tied previous to intraperitoneal injection and they, with Muscatello (5), are the chief advocates of lymphatic absorption. Haidenhain (6), Cohnstein (7), Hamburger (8), Starling and Tubby (9), and others have advanced good evidence of absorption via the blood stream and lately Dandy and Rowntree (10) have shown that, after intraperitoneal injection of phenolsulphonphthalein the dye rapidly appears in the blood and in the urine while lymph from the thoracic duct contains little or none of the dye. They draw the very logical conclusion that the haemic route is the important one for the

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absorption of fluids and that the importance of the lymphatic vessels in this function has been vastly overestimated.

If a peritonitis is induced by injecting suspensions of a granular foreign body into the peritoneal cavity large phagocytic cells may be recovered from the blood of the portal vein which contain in their cytoplasm phagocytized granules of the intraperitoneally injected material. That these are none other than the "pyrrhol cells" or "makrophages" which have been described in animals stained vitally with vital azo dyes is at once evident on examining the portal blood of a trypan blue stained animal after the induction of a foreign body peritonitis with cinnibar or filtered India ink. Smears from the portal vein blood of such an animal show numbers of pyrrhol cells in whose cytoplasm cinnibar or India ink granules may be seen sharply contrasted with the segregated masses of the blue dye.

The presence of such cells in the portal vein is sufficient to direct attention to the possibility of the removal of foreign particles from the peritoneum through the omental blood vessels. The connective tissues of the omentum which fill the meshes of its vascular network are very rich in these wandering phagocytes and there are large masses of them, the so called "taiches laiteuse," in close association with capillary knots or glomeruli. Because of this close association it is not difficult to imagine pyrrhol cells laden with foreign material as penetrating the walls of some of the omental veins and being carried into the portal circulation into which those veins drain.

By far the most remarkable feature noticeable about the portal blood, however, is the presence in it of large quantities of ink or cinnibar which are not enclosed in cell cytoplasm, but which are perfectly free in the blood plasma. Most of this foreign material is in the form of fine granules, but in some cases the ink or cinnibar may be seen in masses which represent aggregates of the finely divided particulate matter in suspension.

Sections of the livers of these animals show pictures which differ according to the length of time which elapsed between the injection of the foreign body into the animal and its execution. The livers of animals killed a short time after the injection are apparently normal, save that the phagocytic endothelium contains varying amounts of foreign pigment which has come from the peritoneal cavity. The portal vein and the hepatic capillaries contain numbers of phagocytes, which have in their cytoplasm, besides the vital dye with which they were previously colored, the granular material which they have engulfed

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from the intraperitoneally injected suspension. These vessels also contain numbers of granules and aggregates of cinnibar or India ink.

If the animals have been allowed to live 24 hours or more after the injection of cinnibar, the liver shows constant abnormalities. That there is still an influx of granular material coming from the peritoneum is evident from the presence of fine granular material and macrophages in the portal veins and the capillaries. The liver is studded throughout with numerous foreign body giant cells of huge size, some of them having fifty or more nuclei whose cytoplasm is stuffed full of phagocytized cinnibar and vitally stained with trypan blue. The portal veins are dilated and full of blood almost to bursting, as though we had to deal with a partial blocking and a passive congestion. On the other hand, the intralobular veins are found dilated or empty, save for a few red blood cells and leucocytes, among the latter being some free pyrrhol cells usually with cinnibar inclusions. We cannot at this time discuss the origin of these giant cells. Whether they are all formed from the phagocytic liver endothelium, or whether immigrated macrophages which have been caught in the liver capillaries have any part in their formation, we cannot say at present.

While these findings are suggestive of the absorption of granular materials by way of the blood stream and point to the omentum as the organ where this absorption takes place, they cannot be accepted as proof of fact, since there is no way of excluding the diaphragmatic lymphatics from participation in the removal of substances from the peritoneum and the entrance of granular material into the circulation via the thoracic duct. For these reasons it was necessary, in order to establish absorption by the omentum beyond question, to bring that organ into contact with solutions and suspensions in such a way that foreign material could not find its way to other absorbing surfaces. This was accomplished by drawing the omentum out of the body of the animal through a midline incision and immersing it in the fluid which we wished to study. Participation of the lymphatics in the absorption of the material was eliminated by preliminary ligation of the thoracic duct. Since these experiments must of necessity run for several hours in many cases, the difficulties of the anaesthesia problem are obvious and we finally came to use animals upon which the operation of decerebration had been performed. Decerebrate animals are ideal material for such experimentation since they lie motionless and rigid with regular pulse and respiration, and blood pressure which is more nearly normal than that of any anaesthetized animal. The omenta of animals pre-

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pared in the manner described above were immersed in true solutions, in pseudo solutions of high molecular dyestuffs like trypan blue, in colloidal metals, and in filtered India ink. After exposure for varying lengths of time, the animals were killed and their tissues examined.

Up to the present time little has been known about the organs concerned in the removal of fluid from the peritoneal cavity and the previous deductions about the vascular path of removal have been based almost entirely upon pharmacological studies, e.g., the work of Melzer, and others with strychnine solutions and that of Dandy and Rowntree on the absorption of phenolsulphonphthalein. Except for the experiments of Rubin (11) on peritoneal absorption in animals whose omenta had been resected there is no experimental evidence which indicates the omentum as a fluid absorbing surface.

In these studies on the absorption of solutions by the isolated omentum we have for the first time attempted to use a histological method of attack in localizing the absorbing surfaces of the peritoneum by exposing the omentum to solutions of potassium ferro cyanide and iron ammonium citrate and fixing the omentum, liver, kidneys, lymph glands, etc., of the sacrificed animals in acid formalin. It was hoped that a study of the distribution of the resulting precipitates of Prussian blue would throw some light on the route of removal of the original fluid and the mechanism by which it found its way into the channel of escape. The citrate ferro cyanide solutions were exactly isotonic with the blood plasma so that any interference by a difference in osmotic pressure was negligible. It may be well to emphasize here the fact that the pressure on the omentum and the fluid to be absorbed—the intra-abdominal pressure—was lowered by exposure of the omentum to the air. This is far from being the case when studies on absorption are made by injecting fluids intraperitoneally. Even small amounts of fluid have a tendency to raise the intra-abdominal pressure and the tremendous rise in pressure which must follow injections of the large volumes of fluid used by some workers cannot but materially affect the mechanism of absorption and drainage.

True solutions and pseudo solutions are absorbed through the blood vessels of the omentum very rapidly. The kidneys of animals killed three hours after immersion of the omentum in the citrate-cyanide solution and fixed in acid formalin show macroscopically on section a deep blue color in the papillae and the cortex due to precipitation of the Prussian blue by the acid. Masses of Prussian blue are visible on section about the portal vessels in the liver. Spread preparations of

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the omentum and microscopic sections show the omental veins to be filled with Prussian blue precipitated in granules so fine as to have the appearance of an homogeneous mass when examined with the highest power dry lenses. Finely divided precipitates of Prussian blue can be seen in the cytoplasm of the endothelial cells themselves though the cell nuclei are always free from any granular deposit.

Since Melzer suggests that ligation of the large lymphatic ducts may cause a stasis followed by an increased pressure on the fluid in the tissue spaces and a consequent abnormal flow of that fluid into the blood vessels, these experiments were repeated on animals without ligation of the thoracic duct. The results were identical.

Experiments with trypan blue were especially striking; an hour's exposure of the omentum to a 1 per cent solution of the dye resulted in a very perceptible staining of the animal's skin and mucous membrane.

Granules of particulate material are absorbed along the same channels, since after exposure of the omentum to filtered India ink, carbon granules may be found in the portal veins. The granules absorbed are very small and where masses of ink were found they were evidently aggregates of very fine particles. The aggregates seem most liable to occur if the circulation is for any reason poor or impeded.

The drainage of particulate matter from the serous cavities via the blood stream is most extraordinary and was most unexpected since the blood vessel wall is not credited with permitting the passage of anything but fluids with a possible exception in the capillaries of the intestinal mucosa. It is probable that only the very small granules of ink pass into the omental veins and we hope soon to be able to state more definitely the granular size limits within which this is possible. Microscopic sections of the livers of these animals show that some ink is phagocytized by the capillary endothelium but most is found as free granules in the lumen of blood vessels. In all a considerable amount of granular material must leave the cavity of the peritoneum through omental veins.

In none of our sections or the numerous span preparations which we have made by various methods have we been able to find lymphatic vessels in the omentum in any of our animals and our sections show clearly that even if such vessels exist, omental lymphatics have no important rôle in the drainage of the peritoneal cavity. On the other hand the omentum plays a very large part in the actual drainage of the peritoneal cavity. True and pseudo solutions and granules of particulate material find their way through omental vessels to the organs

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of the body destined for their ultimate reception and storage or destruction and excretion, and the path by which they leave the omentum is not a lymphatic but a haemic one.

In a later communication we shall describe fully the manner in which various substances pass through the blood vascular and lymphatic walls and their fate after their entrance into the blood and lymphatic circulation. Studies are now under way on the exit of true solutions, colloids and particulate substances through the various absorbing surfaces of the serous cavities of the body.

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STUDIES IN BLOOD PRESSURE ESTIMATION BY INDIRECT METHODS

II. The Mechanism of the Compression Sounds of Korotkoff[1]

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The preceding paper of this series (1) concerned itself with the mechanism of the oscillatory method of determining the blood pressure. In the course of that study parallel observations were made on the sounds that can be heard in the artery below the local compression in the hope that information might thus be gained with regard to the origin of the compression sounds of Korotkoff. The outcome was a working hypothesis. In the present paper this hypothesis is elaborated and the experiments devised for the purpose of testing its validity, as well as that of recorded hypotheses, are presented.

SUMMARY OF VIEWS ON THE MECHANISM OF THE KOROTKOFF SOUNDS

In reviewing the views held with regard to the causation of the compression sounds of Korotkoff, it might be well first to call to mind the mechanism of the production of sounds in general and of arterial sounds in particular. Physics teaches that sounds arise from vibrating sources. If the vibrations are regular and of sufficient frequency a musical note is heard. The pitch of a sound is determined by the frequency of the vibrations, its loudness by the amplitude of the vibrations. The frequency of a vibrating membrane or arterial wall bears an inverse relation to the area of the membrane participating and a direct relation to its tension; while the amplitude of the vibrations depends upon the blow struck, which is determined by the magnitude and rate of development of the pressure or tension that starts the vibrations. We owe primarily to Friedreich (2) the demonstration of the fact that arterial

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sounds partaking of the quality of notes are due to sudden changes, either an increase or a decrease, in the tension of the arterial wall, and that murmurs are determined by eddies set up beyond a constriction in the bed of the stream.

The views held with regard to the origin of the compression sounds of Korotkoff, now extensively employed in the estimation of the arterial blood pressures in man, may, for the sake of convenience, be divided into two groups:

a. Views locating the production of sound in an empty artery beyond the compression. Korotkoff is apparently of the view that compression sounds are due to "the forcing apart of the wall (of the artery) by the first stream of blood which reaches the artery below the cuff. He maintains that the lower part of the brachial artery, during the time the compression is exerted above it, is in a condition of complete relaxation and that the first blood stream causes a sudden sharp stretching of the wall with the consequent production of sounds." Korotkoff offers as proof of this contention the fact that a sound is produced when salt solution is poured into the iliac artery of an animal (3). This view or slight modifications of it (4) seem to be held by the majority of those who have devoted some thought to the subject.

b. Views locating the seat of sound production in the part of the artery compressed apparently are to be found only in the very recent literature. In 1914 MacWilliam and Melvin (5) were forced to this conclusion by the observation that the "sounds are perfectly well developed and characteristic" when the *artery* consists merely of a tube in a compression chamber. These investigators ascribe the sound to vibrations determined by a change in the form of the tube in the compression chamber. It might be added here that the changes in form they describe are not those that occur under a pulse of the configuration of the arterial pulse (1). Flack, Hill and McQueen (6), maintain, without however, offering any experimental evidence for their view, that the compressing armlet "converts the compressed area (of the arm) into a resonating mass, the pulse is not damped down in the labile arteries, but strikes the blood which fills to distention, not only the main artery, but every patent arteriole throughout the mass, and causes the whole tense mass to vibrate."

Von Frey's explanation of the fact that when the arm is plunged vertically into a dish of mercury the shock of the pulse can be felt with special distinctness at a very definite point, is of interest in this connection. He is of the opinion (7) that the pulse is felt most distinctly

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at that place because there the blood flow is checked, the pulse waves are reflected positively, and the summation that results gives to the waves sufficient energy to produce a sensible pulsation. It may, however, be said now that this explanation cannot be the correct one, for if it were, the sensation caused by simply occluding the artery should be as intense as the sensation that is experienced when the compressing pressure lies between the systolic and diastolic level. As is well known, this is not the case.

Gittings (8), while accepting the view with regard to sound production prevailing at the time he writes (1910), believes that the compression chamber contributes to the sound by virtue of its action as a resonator. This conclusion he bases upon the observation that the sounds are much louder when the artery is compressed with the usual pneumatic cuff than with an Esmarch bandage.

THE NEW HYPOTHESIS

The investigation of arterial sounds which forms the basis of this paper forces us to the conclusion that the main mechanism of the compression sounds is as follows: Under compressions which permit the pulse to determine relatively wide excursions of the arterial wall in the compression chamber, that is, under compressing pressures ranging from systolic arterial pressure to, and even a variable distance below, diastolic pressure, the volume of the compressed artery increases abruptly with each pulse (1). This permits a considerable volume of blood to enter the opening artery with a high velocity. The motion of this column of blood is, however, suddenly checked where it comes into contact with the stationary, or practically stationary, column of blood filling the uncompressed artery below. The water hammer that is thus set into play distends the arterial wall at the point of impact with unusual violence. This distention sets the arterial wall into vibration and the sound is produced.

Water hammer may be defined as the pressure exerted when the motion of a mass of fluid is more or less suddenly checked. Some idea of the powerful effects of water hammer is gained from the statement (9) that the rise in pressure caused by suddenly stopping the flow of water in a pipe may be so great as to cause the bursting of the pipe. The following crude experiment, made under mechanical conditions that resemble somewhat those obtaining in blood pressure observations, will serve to indicate the magnitude of the forces we have to deal with in the present problem. A pressure bottle was connected with a

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mercury manometer by rubber tubing of 7 mm. bore through a maximum valve. The whole was filled with water and the bottle was elevated until it exerted a pressure of 140 mm. of mercury on the manometer. The rubber tube was then compressed close to the valve by grasping it between the finger tips, held close together, and the ball of the thumb. This was done in such a way as not to alter the pressure indicated by the manometer. Then the compression was released as quickly as possible. The rate of decompression thus produced is probably somewhat faster than the rate of rise of pressure determined by the arterial pulse. With the decompression, the momentum of the water rushing to fill the tube as it opened drove the stationary mercury of the manometer 40 mm. above the level sustained by the head of pressure of the bottle. It is not without interest to add that at the same time a sound can be heard by listening over the tube with a phonendoscope.

The factors at work in developing the maximum pressure exerted by a water hammer are given by Joukowsky (10) in the following formula:

$$P = \frac{\lambda V w}{g} \qquad (1)$$

where

P = water hammer pressure (in excess of the static pressure),
λ = the velocity of wave motion,
V = the extinguished velocity of the fluid,
w = the weight of a cubic unit of the fluid, and,
g = the acceleration due to gravity.

If the conditions obtaining in blood pressure observations were as simple as those the hydraulic engineer has to deal with it would not be difficult to determine more or less accurately the values for the factors of Joukowsky's formula. The problem of the physiologist, however, is complicated by the fact that he has to do with a pulsatile instead of a steady progression of fluid through tubes which, instead of being rigid, though elastic, distend markedly under pressure and collapse under compression; that the flow is checked not by a rigid valve but by a column of stationary, or practically stationary, fluid contained in a tube of variable bore.

For the present we are interested only in those factors of the formula that would vary in the course of a blood pressure observation; they are λ and V. For present purposes therefore

$$P = \lambda V. \qquad (2)$$

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From Tigerstedt (11) we obtain the following formula for λ:

$$\lambda = k\sqrt{\frac{g\,e\,a}{\Delta\,d}} \qquad (3)$$

in which

g = the acceleration due to gravity,
e = the coefficient of elasticity,
a = the thickness of the wall of the tube,
Δ = the specific gravity of the fluid,
d = the bore of the tube, and,
k = a constant.

It is obvious that in a blood pressure experiment, practically the only variables of this formula would be the coefficient of elasticity, the bore of the artery and the thickness of the wall. Grünmach (12) has shown, in the case of artery, that the effect both of bore and thickness of wall is almost negligible relative to the effect of the coefficient of elasticity, and we actually know that both the coefficient of elasticity and the rate of transmission of the pulse increase with the distention of the artery. We may therefore conclude that during decompression of an artery the factor, λ, of formula *2* tends to increase.

It is much more difficult to derive the extinguished velocity, V. It will depend on the velocity the blood has attained in the compressed segment of artery at the moment of the impact with the column of blood below, and upon the degree the column of blood in the artery below checks this motion. It is practically impossible to obtain the absolute values of these factors. Discussing first the velocity at the time the blow is struck, it seems justifiable to assume that resistance to flow will cause a short though measurable time to elapse before the blood can traverse the length of the compressed segment of artery as it is opened by the rising pressure of the pulse. The velocity through the compressed segment would therefore tend to increase as, during decompression, the walls of the artery are more and more readily pressed apart. Another factor probably exerting an effect upon velocity is the difference between the compressing and the arterial pressures at the moment the blow is struck. If no time were required for the blood to traverse the length of the compressed segment it is obvious that there would never be any pressure difference. If, again, the time required to traverse the artery were brief and constant throughout decompression then, since the earlier parts of the anacrotic limb of the pulse wave rise more rapidly than the later parts, the force

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driving the blood, and consequently the velocity of flow, would increase with decompression. But it is during the later phases of decompression that low resistance to the flow would tend to shorten the time required to strike the blow, and so diminish the effective difference between compressing and arterial pressures. We find, therefore, that what seem to be the two main factors influencing velocity so act during decompression as to oppose each other. Obviously it is difficult to reach a satisfactory conclusion with regard to the effect of lowering the compressing pressure upon the velocity of the initial flow in the compressed segment by a priori considerations alone.

To know the value of V in formula *2* it is still necessary to ascertain the degree to which the column of blood below checks the flow. It seems fair to conclude that the factors checking a moving column are essentially similar to those that affect the water hammer phenomenon in general; the forces acting to suddenly check a moving column are also the very ones that would oppose an effort to suddenly set a stationary column into motion. It would therefore seem that the suddenness with which the moving column is checked will vary mainly as the coefficiency of elasticity of the lower part of the artery. The checking effect will therefore increase as the lower artery distends. It may consequently be concluded that V of formula *2* increases during decompression.

Since both λ and V of formula *2* seemingly increase as the compressing pressure falls from the systolic to the diastolic level, we may conclude that water hammer action likewise increases.

Estimated velocity of flow in the dilating artery. The first paper of this series furnished data indicating the existence of conditions compatible with water hammer action during blood pressure observations. It was there shown that as long as the compressing pressure exceeds the diastolic pressure the artery is closed, or practically so, during a part of each pulse cycle. This means that the column of blood in the distal artery is stationary, or practically so, in the part of the cycle immediately preceding the arrival of the pulse. It was also shown that during the systolic-diastolic period of decompression a considerable volume of blood (as indicated by the volume of the compression pulse) descends with each pulse to fill the collapsed artery. If we assume that the artery fills just to its undistended bore with the particular systole that terminates the first rapid increase in pulse volume, as seen when the compressing pressure falls below the systolic level in records of the volume of the compression pulse [eighth pulse Table IV, fig. 18 (1)],

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we may regard the volume increase per pulse at this time as the minimum quantity of blood that will, at all lower compressing pressures down to the diastolic level, enter the artery as it opens. On this basis it is possible to calculate the bore of the artery at this time and from the bore the velocity with which the first column of blood enters the artery. From information obtainable in figure 18 and Table IV of the preceding paper (1) it can be calculated that the systolic increase in volume with pulse no. 8 amounts in round numbers to 0.2 cc. and that the increase is practically completed in 0.09 second. The bore of the artery at this time is therefore $\left(\text{area} = \frac{\text{volume}}{\text{length}} = \frac{0.2 \text{ cc.}}{5 \text{ cm.}} =\right) 0.04$ sq. cm. and the velocity of flow $\left(\text{velocity} = \frac{\text{volume}}{\text{area} \times \text{time}} = \frac{0.2 \text{ cc.}}{0.04 \text{ sq. cm.} \times 0.09 \text{ sec.}} =\right)$ 55 cm. per second. How rapidly this column of blood moves can best be realized by comparing it with the probable mean velocity of flow in the brachial artery of man. On the basis of data obtained from an article by Hewlett and Van Zwaluwenburg (13) we estimate that the former is at least twenty times the latter. Is it not probable that the impact of a column of blood moving with this velocity against a stationary column of blood will so distend the artery as to set it into vibration?

Preliminary correlation of the Korotkoff sounds with the water hammer hypothesis. Be this as it may, it is interesting to correlate the phases of the Korotkoff sounds with water hammer action as we believe it manifests itself during the gradual decompression of an artery: (a) The first sound is heard at the instant the blood in the artery below the compression chamber shows a brusque acceleration with each pulse (1). (b) The intensity of the sound then increases through the second and third phases[2] as long as the diminishing compressing pressure still suffices to occlude the artery during a part of diastole. It is during this stage, we have shown above, that the energy liberated by the water hammer presumably is constantly increasing. (c) The sudden dulling and weakening of sound (fourth phase) occurs exactly at the instant the compressing pressure leaves the artery open during diastole (1). On the basis of the water hammer hypothesis this weakening is due not alone to a diminution in the velocity of flow into the compression chamber during systole but perhaps even more to the fact that the

[2] The special sounds of the second phase will not be considered for the present.

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column of blood below is no longer stationary at the time it receives the impact, but is moving continually in the direction the impact tends to drive it. (d) Below the diastolic level of compression the sounds usually soon fade away (fifth phase). Even at this time, however, some water hammer action must still persist; for the artery in the compression chamber still increases in volume with each pulse more than does the uncompressed artery. The more rapid flow thus permitted in this part must be checked by the more slowly moving blood below. It is scarcely necessary to add that in those instances in which the pulse itself is brusque enough to elicit sound vibrations from the artery, the sounds will persist even when the artery is relieved of all compression.

The remainder of this paper is devoted to an exposition of the evidence proving this hypothesis and of the objections to other views that have been proposed to explain the Korotkoff sounds.

METHODS IN GENERAL

For the most part the experiments have been performed on dogs. The size of the animal was not of any particular consequence provided the legs were sufficiently long for our purpose. Morphine and ether were employed as anaesthetics in all cases. The ilio-femoral artery is dissected out from well up in the abdomen down to the origin of the *arteria saphena* and all of the branches are tied with fine silk close to their origin. In this way a long, perfectly straight, and unbranched artery is obtained. On the artery thus prepared the compression chamber (our arteriograph) (14) and the stethoscope are placed.[3]

The phonendoscope was used only for special purposes. This instrument can not be applied to the artery without partly compressing it and thus not alone interfering in an uncertain way with blood flow, but also tending of itself to sound production beyond. There was, however, still another reason for not using the phonendoscope in certain phases of the work. Owing to its extreme delicacy it may pick up sounds originating some distance from the spot to which it is applied. The dog's artery emits a sound when it is not compressed. Therefore the artery central of a region of complete occlusion emits a

[3] Never having had any difficulty with this arteriograph we are at a loss to understand Warfield's (15) lack of success with it. The apparatus finally used by Warfield in its stead, on account of its bulk and the necessity of holding it in place by hand, was wholly unsuited to our purposes.

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sound. Under certain circumstances this sound is picked up by the phonendoscope even when it is applied over the artery below the occluded region. Furthermore, when the compression is such as to cause loud Korotkoff sounds, these may be heard by the phonendoscope over a very wide range; not alone over the artery below the arteriograph, but also over a wide zone of tissue to either side of it. It can be readily understood how the detection by the phonendoscope of sounds transmitted such distances from the place of their origin might, under certain circumstances, give rise to difficulty.

When there was danger that this property of the phonendoscope might give rise to confusion, we had recourse to an *artery stethoscope*. The bell of this stethoscope was of the usual form but was closed below by a plate which could be screwed to the bell. The artery passed through the stethoscope by two holes on opposite sides, bisected by the plane of junction of the plate with the bell. This stethoscope was used in all observations excepting where it is specifically stated to the contrary. The arteriograph and stethoscope are trued on the artery so that the latter passes accurately through the orifices of both and so that at no point is the artery pressed upon or bent, and both instruments are then rigidly fastened in place. The arteriograph is then connected by tubes with a mercury manometer, a sphygmomanometer or other recording mechanism, and with an inflating bulb. The stethoscope, for reasons which will be made clear later, was provided with an adjustable side opening.

In our study of the sound phases, we have placed our main reliance in the auditory method rather than in some method of recording sounds. It would seem that none of our recording mechanisms will do just what was demanded by this investigation. While for the exact determination of time relations a record is indispensable, changes in intensity or quality of sound often are not clearly indicated by the microphone. In the present case this difficulty is magnified by the fact that in the dog the sound never wholly disappears with decompression. The dangers of relying entirely upon the graphic method of recording sounds are illustrated by the experiments of Hooker and Southworth (16). These investigators employed the method of Einthoven and Geluk to record the sounds. In order to get only *sounds* by this method they found it necessary to adjust a side opening until deflections of the galvanometer were obtained only in the range of the pulses that produced audible sounds. Are we not to infer from this that the telephone transmitter is affected not alone by sound waves but also by

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pressure waves, and that the adjusting of the side opening resulted in reducing the effect of the pressure waves until only those that were sufficiently strong to cause the artery to emit sounds also moved the diaphragm of the transmitter? Be this as it may, it is obvious that in the present experiments no such adjustment of a sound recording device was possible because there is no lower limit to sound production.

Nevertheless, in order to be in a position to determine time relations, we have in many of our experiments recorded sounds. The method employed, though a well-known one, we happened upon by chance. In some of our experiments a record was desired of the pulse passing through the arteriograph. As we wished to get this pulse before any of it was lost in transmission and as it was also necessary to attach the stethoscope as close as possible to the arteriograph, it was decided to arrange a sphygmograph within the stethoscope itself. The sphygmograph consisted of a receiving tambour connected with a delicate Frank mirror capsule. A channel in the floor of the stethoscope, of about the same width as the artery and covered with very delicate rubber dam, served as the receiving tambour. The channel paralleled the artery. The level of the rubber head covering it was such with respect to the lateral openings of the stethoscope that the artery in the stethoscope just rested lightly on it. It was found that this sphygmograph recorded not alone the pulse in the artery, but also most of the sound vibrations of the arterial wall. With this apparatus, therefore, the arterial pulse and certain of the arterial sounds may be recorded photographically while at the same time the sounds may be followed with the ear. It should be added that the pulse-recording part of this instrument was not delicate in the sense that it would detect the feeblest of pulses. As a matter of fact it often failed to indicate the very earliest of the pulses, pulses that could be faintly felt by the finger.

It has been stated that the stethoscope was provided with a lateral opening the size of which could be readily varied. This was provided for two reasons. In the first place, it was feared that the changes in the size of the artery with the pulse might so affect the pressure on the ear drums as to modify the quality or the loudness of the sounds heard. All danger of any such modification is eliminated by using the instrument with the lateral tube wide open. And in the second place, compression of the artery in the arteriograph is associated with variations in the calibre of the artery passing through the stethoscope. These variations are accompanied by variations in the width of the space around the artery where it enters and leaves the stethoscope. Such

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variations might determine variations in the intensity of the sounds heard. They could be obviated by leaving the side tube open. The attempt was also made to eliminate them by filling with vaseline the crevices between the artery and the orifices of the stethoscope.

RESULTS

Description of the compression sounds

The sounds heard through the artery stethoscope while the pressure in the arteriograph is falling from a high to a low level are in all save one respect usually the same as those heard in man. With a sufficiently high pressure there is no sound whatever perceptible. If the pressure is permitted to fall slowly and steadily, a point is reached where a clear sound is heard, faintly at first, though distinctly, and rapidly increasing in intensity to the characteristic pistol shot sound. Then the sound becomes murmurish in quality, though the murmur is very distinctly accented at the beginning. Soon the murmur gives way to clear sounds which may become exceedingly intense and ringing. These intense sounds last for a short while and then become dull and fainter, rapidly at first, and then more and more slowly. The fainter sounds persist, however, even when there is no pressure whatever on the artery. Not infrequently a phase in which the sounds have a murmurish quality is lacking. All of these sounds, in properly prepared animals, are quite as loud as, indeed often louder than, those heard in man.

The photographic record of the sounds as obtained with the modified stethoscope bears out the ear as regards both intensity and pitch and in addition gives, roughly to be sure, the actual rate of vibrations, from which the position of the sounds in the musical scale can be determined.[4] It should be added that even in especially clear records the fainter sounds, namely those of the early first and later fourth phases, usually do not record. It is not to be expected that the pitch

[4] If proof is needed that the vibrations recorded by the tambour of the stethoscope are actually sound vibrations, it is furnished by the fact that the vibration rate varies in agreement with the changes in pitch as determined by the ear; that the variations in amplitude agree with the changes in intensity, excepting where marked changes in pitch tend to mask the effect intensity would otherwise have on the amplitude of the recorded vibrations; that the vibration period of the Frank mirror capsule differs from the rates recorded (by the Frank method it was 84 d.v. per second); and that vibrations are sometimes recorded when the apparatus receives no impact that could cause it to manifest its inherent period.

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of the sounds will always be the same even in the same animal let alone different animals; nevertheless in our experience it has been fairly uniform. The rate of vibrations of the last of the third phase sounds has usually been 170 to 180 d.v. per second, that is to say, approximately f (172 d.v.) in the octave between c and c'. The first phase and early fourth phase sounds are distinctly lower in pitch; they are usually made up of vibrations at the rate of about 133 per second, and therefore closely approximate c (128 d.v.).

We have gained the impression that other investigators have not been so successful in the use of the dog's artery for the study of compressions sounds. Thus Lang and Manswetowa (17) state that the auscultatory method cannot be used on the leg of the dog. Warfield (15) attempted to differentiate the sound phases in the dog but found it difficult to do so on account of the faintness of the sounds. Again MacWilliam, Melvin and Murray (18) say that "in the case of small animals, the testing of the auditory method is not satisfactory." They evidently found it necessary to use the sheep as the subject of their experiments. By our method the sounds are loud and the phases easily distinguishable in animals no larger, for instance, than the fox terrier.

It has been stated above that in the dog the uncompressed artery always emits a distinct though faint and dull sound. Warfield (15) has also heard a sound in the uncompressed dog's femoral, though not constantly. It should be added that the sound we have heard is not the result of the preparation to which the artery is subjected, for it can be clearly heard through the tissues in the normal, unoperated animal. It might be noted here that the uncompressed dog's carotid artery, as well as that of man (19), normally emits two sounds. The origin of these sounds is still a matter of conjecture. Rarely the uncompressed femoral artery of the dog also emits two sounds. This has not, however, occurred sufficiently constantly to allow of an investigation of the phenomenon.

Inasmuch as the uncompressed femoral artery of the dog usually emits a sound it might be suspected that the first sound heard while decompressing is transmitted from above and does not originate in or below the compression chamber. All of the evidence is, however, opposed to this view. In the first place the qualities of the two sounds are entirely different. These differences in quality are not due to the fact that the normal sound is emitted by a full artery and the first Korotkoff sound by a relatively empty artery. For if the artery is occluded below the stethoscope and consequently remains full at all times the first phase

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sounds still differ in quality from the sound that is heard when the compression pressure is abruptly dropped to zero while the artery remains occluded below. More to the point, however, is the fact that during the early sound phases the sound emitted by the artery above the arteriograph is earlier in the pulse cycle than the Korotkoff sounds. To demonstrate this time relation it is merely necessary to listen to the sounds that can be heard through the phonendoscope applied to the artery below the arteriograph. When the compression pressure exceeds the systolic pressure a sound can often still be heard. This sound is, as has been said, transmitted through the tissues and picked up by the delicate phonendoscope. Now when the sounds are in their first phase, the characteristic Korotkoff sound is heard to distinctly follow by a perceptible interval the sound coming from above.

The bare artery suffices for sound production

The fact, brought out in the preceding section, that the compression sounds obtainable from the bare artery of the dog are perfectly characteristic and rival in intensity those obtainable by the usual method in man, demonstrates conclusively that any other conditions than those obtaining in the present experiments are not essential to the production of the Korotkoff sounds. The contention of Hill and co-workers (6) that all of the compressed arteries "big and small" and "every patent arteriole" under the armlet participate in the production of the sound and that the "whole range of sound is dependent on the resonating effect of the vessels and the tissues surrounding the artery" in the light of this observation loses much of its force. Nor is it necessary to assign any significance, as do these authors, to the presence in the compression chamber of a "tense resonating mass of tissue" that the "impact of the systolic wave" sets into vibration. In the absence of any experimental evidence that these are essential factors in sound production we are justified for the present in concluding that the presence of a large artery of a living animal in a compression chamber furnishes all of the conditions necessary for the production of characteristic Korotkoff sounds.

Is resonance of the compression chamber a factor?

It has been suggested by Gittings (8) that the compression chamber, by acting as a resonator, materially enhances the sounds produced by the tensing of the arterial walls just without the compression chamber. In this connection MacWilliam and Melvin (5) found that the sounds

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"are quite well marked" when the compression chamber and connecting tubes of a pulsating schema are filled with liquid. They therefore set aside resonance as a factor in the mechanism of sound production. This conclusion is not, however, justified by their experiment, for apparently they did not determine whether filling the compression chamber with water *modifies* the sounds; not even Gittings maintains that resonance is the sole factor.

For the purpose of determining whether resonance is a factor we have compared the sounds yielded by a compression chamber which might resonate, with those yielded by the same compression chamber so modified that it could not resonate. The compression chamber in these experiments consisted of the arteriograph, a one litre glass bottle and connecting tubing, all air filled. In order to abolish the resonance of the arteriograph it was, at the desired time, completely filled with water; and in order to effectually prevent any such resonance as the other parts of the compression chamber might possess from acting back upon the artery, a fairly tight plug of cotton wool was inserted into the neck of the arteriograph just above the level of the water in it. This plug, it is needless to say, did not interfere with the rapid equalization of pressure throughout the compression chamber.

With this apparatus it was found that as a rule filling the arteriograph with water, and thus eliminating its resonance, did not change appreciably either the quality of the arterial sounds or their intensity, as heard while the arteriograph was air-filled. It should be borne in mind that these observations can be made only qualitatively, as a considerable interval of time elapses between the successive determinations. Occasionally, filling the arteriograph with water seems to suppress the earliest of the first phase sounds. Inasmuch, however, as the same result is obtained by merely reducing the size of the air space in such a way as not to alter its resonance (see below), it seems justifiable not to attribute this reduction of sound to the elimination of resonance; especially in view of the fact that a perfectly satisfactory explanation of this reduction is found in the retarding effect the water exerts, by virtue of its inertia, on the initial velocity of the blood entering the opening artery.

Influence of the size of the compression chamber on sound production

So far as we have been able to determine, Gittings bases his belief that the compression chamber acts as a resonator merely upon the observation that when the compression is effected by means of an Es-

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march bandage the arterial sounds are either absent or very faint. The same result is obtained, it should be added, when the artery is compressed with the thumb (5). This observation is open, however, to a wholly different interpretation. The Esmarch bandage exerts upon the artery a compression which, compared with that exerted by the air chamber of a sphygmomanometer, has a very low grade of compressibility. With the advent of the pulse, the artery cannot open out to the size it attains in a chamber of high compressibility. The velocity of flow and the rate of transmission of the pulse, two of the important factors determining water hammer action, will therefore be diminished.

The effects on the Korotkoff sounds of limiting the compressibility of the compression chamber have been investigated by inserting a glass stopcock in the course of a vertical glass tube connecting the arteriograph with the manometer and bottle of the apparatus just described above. By turning the stopcock the size of the compression chamber could suddenly be changed from that of the arteriograph alone to that of the arteriograph plus bottle and connecting tubes, or vice versa. A large (1 litre) bottle was used in these experiments so as to render possible a wide range of compressibility. The phase of the pulse cycle in which this change was effected might have been, though it was not, determined in this particular set of experiments. In another series of observations, however, in which the arteriograph was filled with water up to about the level of the stopcock, so as to make possible an extreme diminution in the compressibility of the compression space, the phase of the pulse cycle in which the compression chamber was made small could be determined by noting the level at which the pulsating water in the vertical tube was caught by the closing of the stopcock. In the case of the water-filled arteriograph, it will be noted, the compressibility of the compression space when the stopcock is closed is limited practically to a very slight bulging of the rubber membrane out of the orifices of the arteriograph. It should be added that in order to eliminate any effect that might possibly be exerted through changing resonance of the compression chamber, a cotton plug was again inserted in the glass tube just above the stopcock.

Experiments with the water-filled arteriograph. It is convenient to consider first the experiments in which the changes in compressibility were effected by turning the stopcock of the water-filled arteriograph. In this case the changes in compressibility are extreme. As might have been predicted from an analysis, in the light of the water hammer hypothesis, of related data as given in the first paper of this series, the

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result obtained depends to some extent upon the phase of the auscultatory phenomenon, and upon the phase of the pulse cycle obtaining at the time the stopcock is closed. In the early sound phases, that is, while the extra-arterial pressure is still relatively high, closing the stopcock, and thus reducing compressibility to a minimum, at once stops all sound, irrespective of the phase of the pulse cycle in which the change is made. At lower extra-arterial pressures, when the sounds are intense (third phase), closing the stopcock either stops all distal sound or causes it to diminish markedly in intensity. The sound always disappears when the closing stopcock catches the meniscus of the water low in the vertical tube of the arteriograph, that is, when it catches the pulse in its diastolic phase. The loudest of the sounds heard with the reduced arteriograph occur when the stopcock catches the meniscus high in the vertical tube, that is, when the pulse is in its systolic phase.

This enfeeblement of sound, amounting at the higher compressing pressures to a complete disappearance, is attributable on the basis of the water hammer hypothesis mainly to the limitation of the pulsatile increase in volume, and therefore of the velocity of flow, in the compressed artery, resulting from the splinting of the arterial walls by the markedly reduced compressibility of the compression space. This effect is much more marked when the reduction in the compressibility is effected during diastole, probably on account of the tendency on the part of the confined space to hold the artery closed during the entire pulse cycle.

Experiments with the air-filled arteriograph. When the arteriograph is emptied of water, closing the stopcock still diminishes the compressibility of the compression space, but not nearly to the same extent as in the preceding experiments. Owing to differences in the quality and loudness of the sounds in different animals and to differences probably attributable to variations in the size of the artery relative to the arteriograph, it is difficult to give a categorical account of the changes in the sounds resulting from reducing the size of the air space around the artery. The difficulties may be illustrated by giving in tabular form some of the results obtained (see Table I). But despite variations in different experiments the table does show a certain regularity in the results. Thus, reducing the size of the air chamber while first phase, and occasionally even while early second, phase sounds are heard nearly always causes them to disappear. By these means the compressing pressure at which the first sounds appear may be lowered by as much as 15 or even 30 mm. of mercury. The effect of reducing the size of the

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TABLE I

Showing the effect on sounds of reducing the air space of the compression chamber

SOUND PHASE	EXPERIMENT 6	EXPERIMENT 7	EXPERIMENT 9 EARLY	EXPERIMENT 9 LATE
First early	Disappear	Disappear	Disappear	Disappear
First late				Fainter
Second early	Murmur disappears and sounds become snapping and fainter	Disappear	Sometimes louder, sometimes fainter	Sometimes louder, sometimes fainter
Second late	Murmur disappears and sounds become snapping without change in intensity	Disappear(?)	*	*
Third early	Sometimes the murmur reappears; if not, sounds become louder	Sometimes louder, sometimes fainter	No change in intensity	
Third late		No change	Fainter	Always fainter
Fourth	No change	No change	Fainter	

* No second phase sounds.

air chamber during the second phase depends presumably upon the phase of the pulse cycle in which the reduction is made to take place—the sounds sometimes becoming fainter, sometimes louder; sometimes the blowing sound disappears. In the third phase the sounds usually become fainter though they may not be changed, or may rarely become louder; when the second phase is especially well developed, diminishing the size of the air chamber during the third phase may cause second phase sounds to return. During the fourth phase the sounds usually are not appreciably altered; occasionally they become slightly fainter.

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It will be noted that at the higher compressing pressures the results (disappearance or reduction of sound) obtained upon reducing compressibility are qualitatively, though not quantitatively alike with both the water and the air-filled arteriographs. This statement applies also to the effect upon sound of closing the stopcock, presumably during diastole, at all lower compressing pressures. The result of closing the stopcock presumably during systole is, however, wholly different: the sound in the case of the air-filled chamber may actually become louder than that heard while the compression chamber is large. To explain this result we find it necessary to have recourse to an additional factor. At the lower compressing pressures closing the stopcock, while reducing the compressibility of the compression space, has not anything like so marked an effect upon it as at higher compressing pressures. Under such circumstances it is conceivable that the effect the closing of the stopcock in different phases of the pulse cycle has upon the mean compressing pressure may become more significant relatively than all the other effects. It is clear that when the stopcock in closing catches the artery expanded by the pulse, the mean level of the compressing pressure will be lowered slightly; and that when the artery is caught in its collapsed condition, the mean level will be raised: in other words, the compressing pressure in effect is lowered or raised, respectively. The effect upon sound production of closing the stopcock at low compressing pressures, it will be noted upon consulting the Table I, is consistent with the premises. When the stopcock is closed while the compressing pressure lies in a region where, say, a slight *decrease* in its level would cause a comparatively marked change in the intensity of the sound, say, an *increase*, and while, say, systole prevails, the loudness of the sound would increase. And when under exactly the same circumstances the stopcock is closed during diastole the loudness of the sound would *decrease*. If, however, the stopcock is closed again at a time when a slight *decrease* in the level of the compressing pressure causes a *decrease* in sound intensity, the changes in the intensity of the sound would be just the reverse of those mentioned above.

In conclusion it is important to bear in mind that the changes in intensity of sound noted in the foregoing experiments can not be attributed to changes in resonance, the cotton plug just above the stopcock having the effect of keeping the resonating properties of the arteriograph constant throughout the experiment. The effects upon sound production of limiting the compressibility of the arteriograph,

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it will be noted, are similar to the effects of compressing the artery with an Esmarch bandage or with the thumb. Inasmuch as the former are not attributable to resonance changes but rather to a limitation of compressibility, it seems justifiable to conclude that the same explanation applies to the latter also.

Is the artery beyond the compression chamber essential for the production of sound?

The observation of MacWilliam and Melvin to the effect that compression "sounds are perfectly well developed and characteristic" when the phonendoscope is applied over the glass tube issuing from the artery in the compression tube of their circulation schema even when there is no artery beyond, answers this question in so far as it applies to an artificial circulation. We have confirmed this observation in animal experiments. We find that occluding the artery in the lower neck of the arteriograph by thrusting into the latter a nicely rounded glass rod to a depth of some 4 to 6 mm. in such a way as to compress the artery against the side wall of the neck, does not cause the sounds that can be heard by placing a phonendoscope on the arteriograph to cease. We can go further and add that thus occluding the artery fails to alter in any marked degree the character of the sounds heard: they sometimes become slightly louder, sometimes slightly fainter.

In this connection it is necessary to bear in mind the possibility that the part of the artery within the compression chamber forming the transition between the part collapsed, in the middle regions, and the part uncompressed, just at the lower edge of the compression chamber, may be a factor in sound production. MacWilliam and Melvin, we assume, believe they eliminated this possibility by treating the lower part of their arteries with formalin. We do not; however, understand how such treatment succeeded in removing the lower conical closure. It was in an effort to accomplish this that we employed a glass rod and pushed it alongside the artery from without rather than into the lumen of the artery, instead of merely blocking the artery in the usual way at the point where it issues from the arteriograph. Yet we cannot be absolutely certain that even by this procedure we succeeded in completely eliminating this factor; for some blood is bound to be trapped between the compressed and the occluded segment when the artery closes under the compression. It would seem, however, that an objection on this basis to the conclusion that the distal artery

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is not essential to the production of compression sounds would be rather far-fetched.

The fact that the artery beyond the compression chamber is not essential to sound production obviously throws out of court all explanations of sound production based on the injection of blood into an empty artery. On the other hand, it is clear that eliminating the artery beyond does not reduce the factors that determine the magnitude of water hammer pressure.

Does the artery beyond the compression chamber contribute to sound production?

It has been shown that the artery beyond the compression chamber is not essential to sound production. This observation does not, however, preclude the possibility of sound production in the lower artery also; for it is conceivable that the impact that first produces sound may be propagated by the blood in sufficient amplitude to continue to produce sound for some distance along the artery. Indeed it will be demonstrated later that during the louder sound phases the arterial walls for some distance below the compression chamber are stretched unusually sharply by each pulse. The interest that attaches to this question lies mainly in its bearing on those views that limit sound production to the compression chamber. If the Korotkoff sounds are produced alone by a change in the form of the tube in the compression chamber (5), or by "a tense resonating mass of tissue" in the compression chamber which is set into vibration by the "impact of the systolic wave" (6), the sound would be propagated from that place as sound through the blood and the tissues. If so, it should be possible to hear the propagated sound quite as well over, say, a metal tube intercalated in the course of the peripheral artery as over the artery itself. On the other hand, if the sound heard along the course of the artery below the compression chamber is produced by a propagated impact that induces sound as it proceeds, an intercalated, inextensible tube should not give out the same sound as the artery immediately above and below it.

In order to put this question to the test of experiment, the effect on the arterial sounds has been determined of causing a paraffined, metal tube 4.5 cm. long to replace the same length of artery, beginning about 4.5 cm. below the compression chamber. Then the segment of artery between the compression chamber and this tube, the tube itself, and finally the artery just below the tube, were in succession placed in the

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stethoscope. The crevices between the artery and the opening into the stethoscope were filled as thoroughly as possible with vaseline and the side tube of the stethoscope was left wide open. It was found that in the third phase the sounds were loudest close to the arteriograph and faintest over the tube, where the sounds, when present at all, were feeble and distant. Presumably, therefore, we are dealing here, in large part, at least, with a propagated impact that produces sound locally as it proceeds, though to a certain extent the transmission of sound as sound also takes place. It should be added that the phonendoscope detects over the metal tube a fairly loud sound. In view, however, of the delicacy of the phonendoscope, referred to above, it seems fair to assume that in this case it is picking up a transmitted sound.

Configuration of the compression pulse

Records have been made of the compression pulse, that is, of the pressure changes in the compression chamber produced by the pulse, by connecting the arteriograph, through a 0.5 or 1 L. air-filled bottle, either with the Erlanger sphygmomanometer provided with a Frank mirror capsule in the place of the usual tambour, or with a Frank mirror capsule directly. Records have been made both by the method of intermittent escapement, when as a rule the pinhole in the recording tambour was closed, and by the method of continuous escapement. The records made through the Erlanger sphygmomanometer are more ample and therefore have more fling than those made directly through the Frank capsule; this has been an aid in the analysis of the records rather than a detriment, because the presence of a certain amount of fling brings out more sharply changes from one pressure gradient to another.

In the preceding paper these records were analyzed as regards the amplitude of the oscillations, and its relation to the moment the artery during decompression begins to open momentarily with each pulse, and later ceases to close. It was stated in that paper that the first sound is heard presumably the instant the artery opens momentarily, and that the dulling of sound agrees exactly with the pulse with which the artery fails to close. Here we are interested in determining whether the configuration of the individual compression pulse is in agreement with the water hammer hypothesis.

We may first ask, what influence would we expect water hammer to exert on the configuration of the compression pulse? It has been

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shown (1) that the compression pulse can be produced only by volume changes that are extensive enough to compress the air in the chamber to the pressures of the compression pulse. Therefore, to be in a position to answer the above question, it is necessary to know something with regard to the volume changes determined by water hammer. So far as I know, the volume changes produced by water hammer have never been recorded. However, from the theory of water hammer as developed by Russell (9), the volume changes may be derived as follows:

If we regard the column of water as divided into successive laminae, the lamina that meets with the obstruction is stopped, crowds up against it and is compressed by virtue of its own kinetic energy. As it is compressed, the ring of pipe wall surrounding it is distended. While this lamina is being compressed and shortened the next lamina behind it follows on with undiminished velocity, until the compression of the first lamina is complete. It in turn then suffers retardation and compression, at the same time stretching the pipe wall around it. Other laminae follow in succession so that in a very short time the pipe is distended back a considerable distance. In other words, one effect of water hammer is to distend the tube backwards from the point of impact.

Therefore water hammer would distend the artery first under the lower edge of the compression chamber. This distention would, however, extend rapidly back past the upper edge of the chamber where, so far as concerns the compression pulse, distention due to water hammer per se would cease abruptly. Then, provided the vibrations set up in the blood column have ceased, the pressure head of the pulse proper alone would determine further volume changes of the compressed artery. The compression pulse up to the crest of the pulse proper, for the sake of convenience, might on this basis be divided into three periods, namely:

a. A period included between the onset of the pulse and the forcing open of the artery in the compression chamber, during which the upper conical closure would be filling with blood and the pressure in the chamber would therefore be increasing at a rate proportional to the rise of pressure with the pulse.

b. The second period would be coterminous with water hammer as described above. The rate and amplitude of the rise of pressure would presumably be determined by the force of the water hammer. In case the backward distention of the artery reached beyond the upper edge

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of the compression chamber this period of the rise of the compression pulse presumably would be at a more or less uniform rate up to an abrupt termination.

c. The third period would be distinguishable from the second only if water hammer action were completed before the pulse proper had attained its crest. Then there would be two crests, the first, due to water hammer, terminating the end of a rise that is uniform in rate and appearing earlier and earlier in the compression pulse, the second terminating a gradient partaking of the form of the corresponding part of the pulse and appearing at a constant time interval after the beginning of the compression pulse.

Let us then examine a typical record (fig. 1, Table II) with the theory of the volume changes produced by water hammer in mind, assuming for the present that water hammer is active as long as the sounds are louder than those emitted by the uncompressed artery.

1. When the sounds first begin to come through, the only recognizable change in the anacrotic limb of the compression pulse is an increase in amplitude. This is not inconsistent with the hypothesis, for the first opening of the artery during decompression is very brief in duration and occurs so close to the crest of the pulse proper that period *a* (see above) is merged with period *b*, while there is no period *c* distinguishable from period *b*. In other words, all that we could expect at this time is an increase in the amplitude of the pulse.

2. After the compressing pressure has fallen a very few millimeters of mercury below the level at which the sounds first became audible, the compression pulse begins to show a double crest. The first of these crests, which presumably terminates period *b*, soon becomes very distinct and develops into an obvious fling; and it remains distinct until the sounds begin to diminish in intensity, when, within a very few pulse beats, it can be recognized only with difficulty. From the time the first crest becomes well marked, and until it begins to diminish in clearness, the upper part of the rise of pressure that leads to it occurs along a straight line. There is, however, no mark on the rise of the compression pulse that is sharp enough to be of use in distinguishing between the curved line belonging presumably to period *a* and the straight line belonging presumably to period *b*. The first crest is attained earlier and earlier in the pulse as the compression diminishes.

3. It is sometimes difficult to decide upon a point on the compression pulse that can be regarded as its true crest, because this part of

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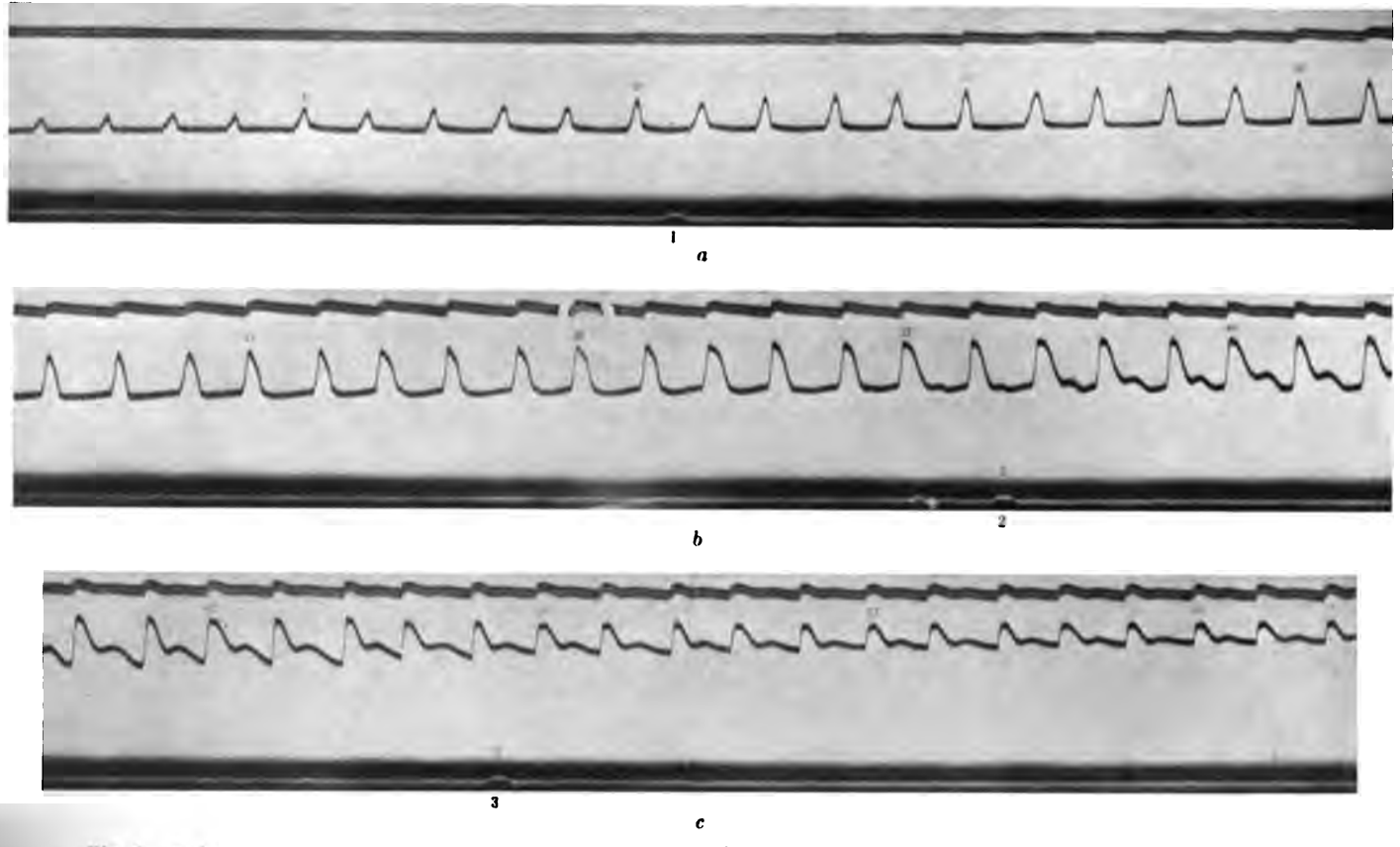


Fig. 1. *a*, *b*, *c*. **Record from the femoral artery of the dog while decompressing by the continuous method. Reduced to ¼ size. Read from left to right. Upper tracing: arterial pulse and sounds just beyond the compression chamber, by the recording stethoscope; middle tracing. compression pulse; lower tracing: time in fifths of seconds and signal. (1) First signal, sound becomes audible. (2) Second signal, sound becomes intense. (3) Third signal, sound becomes dull.**

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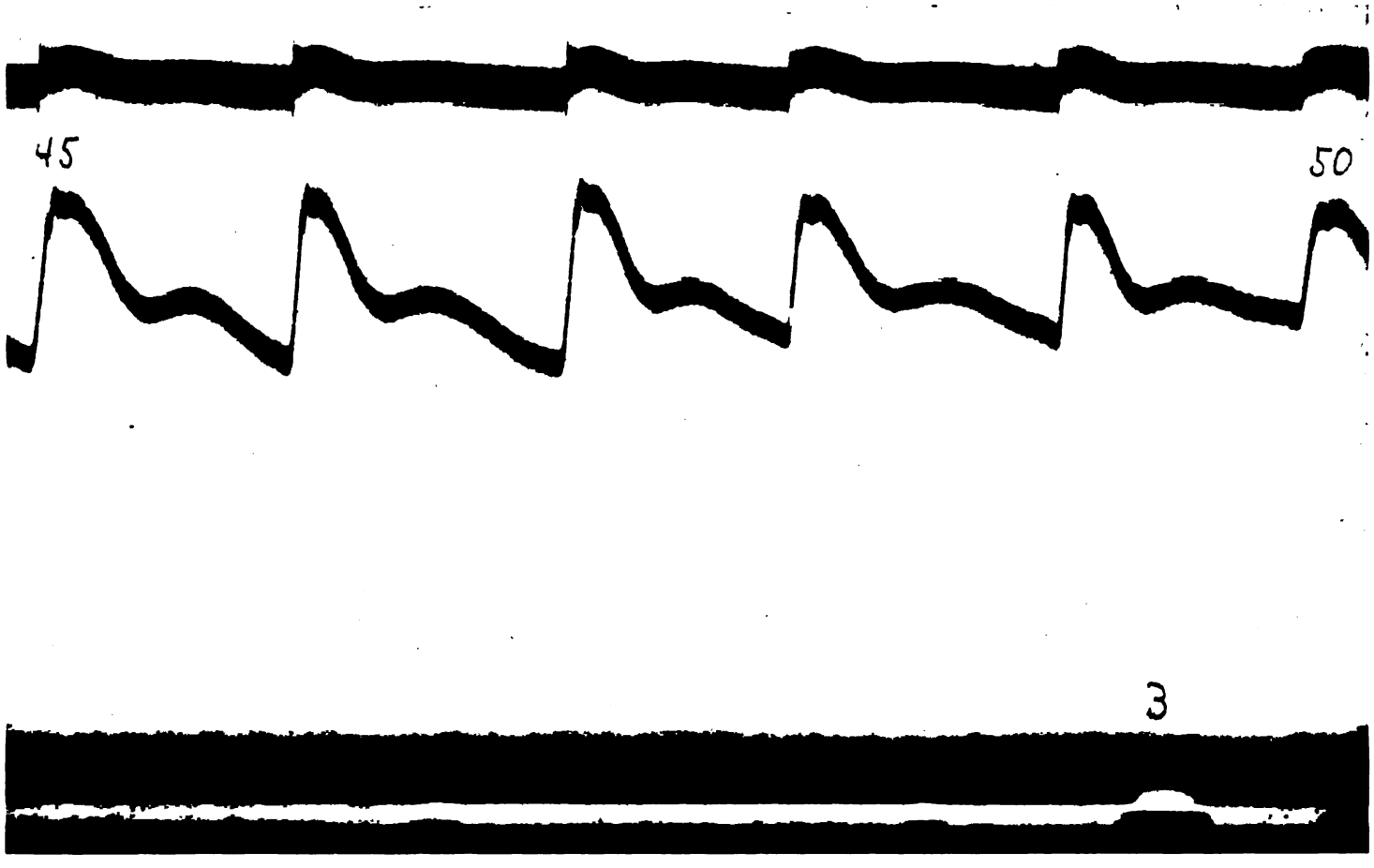


Fig. 2. Section of figure 1 (pulses 45 to 50 inclusive), reproduced in full size to show details.

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TABLE II

Analysis of figure 1

(1)	(2)	(3)	PERIPHERAL PULSE			COMPRESSION PULSE				(11)
NUMBER OF PULSE *	TOTAL AMPLITUDE	DURATION OF PULSE CYCLE†	(4) Time to	(5) Amplitude of first crest‡	(6) Amplitude of second crest	(7) Amplitude of first crest	(8) Time to first crest	(9) Time from sound to top of first crest	(10) Amplitude from sound to top of first crest	RATIO Col. 10 / Col. 9
	mm.	sec.	sec.	mm.	mm.	mm.	sec.	sec.	mm.	
1	4.5	0.53								
2	6.5	0.50								
3	7.5	0.51								
4	6.5	0.53								
5	10.5	0.50								
6	9.0	0.54								
7	11.0	0.54								
8	12.5	0.51								
9	11.5	0.56								
10§	16.0	0.50	0.071		1.0			0.015	2.5	167
11	13.5	0.52			—?					
12	16.0	0.54	0.063	0.5	1.0			0.019	2.5	131
13	17.0	0.50	0.071	0.6	1.2	17.0	0.082	0.02	3.5	175
14	16.5	0.56	0.071		1.0			0.02	3.5	175
15	18.5	0.53	0.060	1.0	1.4	18.0	0.071	0.019	4.0	210
16	18.0	0.51	0.079	1.0–	1.3	17.5	0.097	0.019	3.0	158
17	19.5	0.57	0.059	1.2	1.5	18.0	0.075	0.02	4.0	200
18	20.0	0.51	0.056	1.3	1.4	19.0	0.069	0.02	4.0	200
19	19.0	0.53	0.066	1.4	1.4	18.0	0.082	0.019	3.5	184
20	20.5	0.56	0.053	1.8	2.0	19.0+	0.065	0.019	5.0	264
21	20.5	0.49	0.054	1.8	2.3	19.0	0.065	0.02	6.5	325
22	21.0	0.55	0.048	2.2	2.8	20.0	0.063	0.019	6.5	312
23	22.0	0.54	0.048	3.0	3.4	20.5	0.057	0.018	7.0	389
24	21.0	0.49	0.057	2.2	3.0	20.0	0.075	0.019	6.0	316
25	22.5	0.57	0.043	2.5	3.5	21.0	0.060	0.022	7.0	318
26	22.0	0.49	0.043	2.8	3.0	21.0–	0.060	0.02	7.5	375
27	22.0	0.54	0.046	2.8	3.0	21.0	0.065	0.022	8.0	364
28	23.0	0.54	0.038	3.0	3.2	21.3	0.053	0.022	10.0	455
29	22.0	0.47	0.043	3.0	3.4	21.0	0.057	0.019	8.5	447
30	23.0	0.55	0.037	3.0	3.2	21.5	0.053	0.02	9.5	475
31	23.0	0.49	0.035	3.3	3.3	22.0	0.049	0.02	11.0	550
32	23.0	0.53	0.043	3.3	3.3	21.5	0.057	0.02	10.0	500
33	23.5	0.56	0.032	4.0	3.4	22.0	0.046	0.019	11.5	579
34	23.5	0.47	0.034	3.8	3.5	21.5	0.049	0.022	11.5	546
35	24.0	0.55	0.035	4.0	3.5	21.5	0.053	0.023	12.0	522
36¶	24.0	0.51	0.028	4.0	3.8	22.0	0.046	0.019	12.0	632
37	24.0	0.51	0.038	4.0	3.4	22.0+	0.056	0.018	11.0	611

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TABLE II—Continued

(1)	(2)	(3)	PERIPHERAL PULSE			COMPRESSION PULSE				(11)
NUMBER OF PULSE *	TOTAL AMPLITUDE	DURATION OF PULSE CYCLE†	(4) Time to††	(5) Amplitude of first crest‡	(6) Amplitude of second crest	(7) Amplitude of first crest	(8) Time to first crest	(9) Time from sound to top of first crest	(10) Amplitude from sound to top of first crest	RATIO Col. 10 / Col. 9
	mm.	*sec.*	*sec.*	*mm.*	*mm.*	*mm.*	*sec.*	*sec.*	*mm.*	
38	25.0	0.56	0.026	4.2	3.7	22.0+	0.044	0.019	13.0	684
39	25.5	0.46	0.028	4.2	3.3	22.5	0.043	0.018	13.0	722
40	26.0	0.54	0.026	4.0	3.3	22.0+	0.043	0.019	14.0	737
41	26.0	0.55	0.025	4.0	3.3	22.0+	0.040	0.016	14.0	875
42	27.0	0.48	0.028	4.0	3.3	22.0+	0.044	0.018	12.0	667
43	26.0	0.57	0.025	3.8	3.0	21.5	0.047	0.015	13.0	867
44	26.5	0.49	0.024	4.2	3.3	21.5	0.035	0.015	13.0	867
45	23.0	0.54	0.024	4.0	3.0	19.0−	0.044	0.016	12.5	781
46	23.5	0.56	0.019	4.2	3.3	19.0+	0.037	0.015	15.0	1000
47	24.5	0.49	0.021	4.3	3.3	20.5	0.035	0.016	15.5	969
48**	19.0	0.56	0.013	3.3	3.3	15.0	0.029	0.016	12.0	750
49	18.5	0.50	0.012	3.3	3.3	15.0	0.029	0.019	13.0	684
50	14.0	0.51	0.013		3.4	?		0.022+	10.0	455
51	14.5	0.50	0.010		3.6	?				
52	15.0	0.46	0.012		3.7					
53	13.0	0.49	0.009		3.5					
54	13.0	0.53	0.009		3.6					
55	11.0	0.50	0.008		3.5					
56	11.0	0.56	0.006		3.8					
57	11.5	0.49	0.004		3.8					
58	10.0	0.53	0.003		3.6					
59	10.0	0.54	0.003		3.8					
60	9.5	0.50	0.004		3.7					
61	9.0	0.54	0.003		3.7					
62	9.0		0.003		3.7					

* The mean rate of pressure decrease is 2.2 mm. Hg per pulse; the rate in the vicinity of the systolic and diastolic pressures is approximately 2.5 + mm. Hg and 1.9 − mm. Hg per pulse respectively.

† The time was recorded in seconds. The paper, however, moved very uniformly at the rate of 68 mm. per second.

‡ The amplitude of the sound vibrations is always proportional to this rise.

§ First sound signalled.

¶ Signalled sounds intense.

** Signalled sounds fainter.

†† The figures given in this column opposite pulses 53 to 62 inclusive, were printed incorrectly in the preceding paper (1, p. 431). We are availing ourselves of this opportunity to indicate that another decimal place should be added to the corresponding figures as they appear in that paper in column "Time to peripheral pulse" in Table II, "Dog No. 10."

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the curve not infrequently is made up of a series of waves such as commonly follow the first impact of water hammer. When, however, the records are clear in this respect the second crest continues to appear at approximately a constant interval after the pulse—an interval which is the same as that of the single crest seen in the earliest of the compression pulses. Between the first and second crests the gradient of pressure seems to be determined mainly by the phase of the pulse cycle that is to say, by the head of pressure, that has its course to run at that time.

Relation of the sounds to the compression pulse

In this connection it will be convenient to consider first the general configuration of the phonograms. It has been stated that during decompression the first audible sounds often do not record. In clear records, however, the sound waves appear before the compressing pressure has fallen from 8 to 10 mm. of mercury below the point at which the sounds become audible; at this time the sounds are still in the first phase. The amplitude of the sound waves, as has been said, changes in agreement with the intensity of the sounds as heard. The sound waves occupy the very beginning of the peripheral pulse (see below). In the early sound phases (see fig. 1), the first vibration of each group composing a sound is the highest, the subsequent waves diminishing rapidly in amplitude until they become indistinguishable. Later, when the sounds become intense, it is usually possible to make out a very small sound vibration preceding the peripheral pulse (see fig. 2). This preliminary vibration leads up to the powerful vibrations which are coincident with the beginning of the peripheral pulse. We are inclined to believe that the preliminary vibration is produced by sound transmitted by the blood stream as such, from the point where sound first originates, and that the powerful vibrations represent sound waves started locally within the stethoscope by the arrival there of a transmitted impact. Other explanations are, of course, possible. Assuming, however, that the explanation here offered is correct, it is of interest to attempt to locate by calculation, upon the basis of it, the seat of earliest sound production. The preliminary wave precedes the fully developed wave by roughly 0.005 second. It is, we assume, propagated with the velocity of sound and therefore without any appreciable loss of time. The impact would, however, be transmitted as a pulse wave along an artery that is practically under no tension what-

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ever. The rate with which it is transmitted would therefore be very much slower than that of the pulse under normal conditions. Let us assume that it moves at a rate of 4 M. per second, that is, at about one-half the normal rate. Then the first seat of sound production would be located (400 cm. $= \frac{d}{0.005''}$) 2 cm. above the tambour in the stethoscope. As our apparatus was arranged, this would place the origin of the sound in the lower part of the compression chamber just about where we would expect a water hammer to strike. We have assumed that the first fully developed sound wave marks the same event in all pulses. In order to locate on the compression pulse the point corresponding with the beginning of sound we have, in measuring our records, first determined the relation of the fully developed wave to the compression pulse, and have then carried all points thus located on the compression pulse forward by the duration of the preliminary wave, usually 0.005 second.

The fact that the first fully developed vibration is always the highest and that the sound vibrations succeeding it gradually fade away and have completely disappeared usually before the peripheral pulse has attained its true crest, proves that the sound is produced by a sudden impact in the earliest phase of the rise of pressure that lets the pulse through, and that it is not due to the continued stretching of the arterial walls through the anacrotic phase of the pulse.

If the compression sounds are produced by water hammer they should occur while the water hammer is acting and, in general, their intensity should be proportional to the force of the water hammer. Simultaneous records of the sounds in the artery beyond the compression chamber and of the compression pulse show that this is actually the case (see figs. 1 and 2). The sounds, measured as indicated above, always begin before the termination of the first abrupt rise of the compression pulse where this occurs; indeed it almost seems that they mark the point on the rise where the ascent begins to become straight (see preceding section). In the earlier pulses where the abrupt rise is not clear, the sounds begin a bit before the crest of the compression pulse.

This is exactly in accordance with the requirements of the hypothesis. The very first blood that succeeds in opening up the artery is stopped, distends the artery and produces the sound, while the artery then continues to distend backward. If this assumption is justifiable, we have in the peak at the top of the first rise of the compression

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pulse, an index to the termination of water hammer. The intensity of water hammer would then be measured roughly by the ratio of the amplitude of the part of the compression pulse subtended by these two marks (sound and first crest) to the time elapsing between them. This ratio, given in column 11 of Table II, barring certain fluctuations which are in part due to the wide limit of error of measurement and in part to fluctuations in the circulatory conditions (see below), increases steadily from the time the sounds are first heard until they begin to diminish in intensity. With this diminution, the ratio rapidly falls off, although it can be determined through only a few of the succeeding pulses, the marks of water hammer soon becoming indistinguishable. The amplitude of the recorded sound vibrations varies in general as the magnitude of this ratio (see Table II, col. 5). It might be added that these variations furnish presumptive evidence in favor of the conclusion, reached in connection with the introductory theoretical discussion of the water hammer hypothesis, that water hammer increases during decompression until the artery remains open throughout the pulse cycle.

It has been stated that the water hammer ratio shows some fluctuations. When one examines the phonograms corresponding with the lowest ratios, namely, those obtained from pulses 18, 24, 25, 27, 32, 35, 37, 42 and 45 (Table II and fig. 1; also pulse 45, fig. 2), it is seen that the rate of vibration is invariably slower with these pulses than with any of the others. The amplitude of the vibrations, though, is not very different. Since, however, smaller vibrations of the same real amplitude should, as a result of instrumental error, be recorded larger than quicker vibrations, it seems justifiable to conclude that these particular sounds are lower, not alone in pitch but also in intensity, than any of the other sounds. It is not difficult to show that these sound fluctuations are dependent upon cardio-vascular changes; for the particular compression pulses in which they occur are distinguished from all others by their configuration, being broader across the top. Furthermore, they usually are the shortest pulses in duration, or follow immediately upon the shortest pulses. In the absence of simultaneous blood pressure records it is impossible to determine the exact nature of the cardio-vascular phenomena here transpiring. For present purposes, however, we are alone interested in the fact that, whatever, may be its nature, a cardio-vascular phenomenon that alters the intensity of water hammer action also alters the intensity and the pitch of the compression sounds in the same direction.

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Configuration of the peripheral pulse

The configuration of the peripheral pulse also throws some light upon the nature of the process that produces the sounds. In the present experiments the pulse, it will be recalled, was recorded by letting the artery rest upon the head of a special receiving tambour in the floor of the stethoscope. This method, while not a particularly delicate or constant one, was employed, firstly, because it did not necessitate applying any pressure to the artery and, secondly, because it was desirable to obtain the pulse as close to the compression chamber as possible. Often it was not delicate enough to record the first pulses that came through. In the case of the particular record used here for purposes of illustration (fig. 1) it did actually record the very first pulse that gave rise to a sound.

The first pulses that come through, as has been said, usually give no evidences of sound vibrations, they merely produce small, rounded elevations. When, however, the sound waves become visible, or, at least, shortly thereafter, the level of the record is raised a short distance with the first sound wave. This abrupt rise is followed by a slight decline to a second gradual rise which attains a greater height than the first. Both of these elevations gain in amplitude with decompression, the first, however, more rapidly than the second, with the result that when the third phase sounds are reached the first fully developed sound wave may attain a greater height than the second rounded elevation and it attains this height in the extraordinarily brief period of one-half of a double sound vibration, or approximately 0.002 second. With the onset of the fourth phase sounds the first rise begins to diminish in amplitude while the second elevation continues to gradually increase until with a further fall of compressing pressure, usually of some 5 to 10 mm. of mercury, the pulse has taken on the appearance of a normal wave.

There can be no doubt but that the abrupt rise of the pulse during the loudest sound phases is the result of an impact that is extremely sudden and much more forcible than any the pulse wave of itself delivers, for it may rise to a greater amplitude than the pulse proper. The entrance of the blood under practically systolic pressure into an empty artery in which the pressure may be zero, which is Ehret's explanation of the Korotkoff sounds, fails to account for this rise of pressure above the level of the aftercoming pulse. It might, however, be maintained that the low, rounded waves that come through in asso-

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ciation with the first phase sounds could not come from an impact that is sharp enough to produce a sound. This objection loses much of its force when it is recalled that the quantity of blood that succeeds in getting through the compressed artery at this stage is very small indeed. While it may be sufficient to so distend the artery at the point of impact as to produce a sound, it nevertheless may not be sufficient to propagate a sharp pulse for any considerable distance along the relatively empty artery.

Effects of occlusion of the artery on sound production

When one occludes the artery some distance below the stethoscope in such a way as to leave the artery distended with blood and then observes the sounds in the usual manner while the compressing pressure is falling, the sequence and character of the sounds seem to be but little altered from those heard when the artery is open, except that the murmur of the second phase sounds is lacking. This observation again demonstrates that for the production of the arterial compression sounds in all of their phases, excepting the murmurs, an empty distal artery is not an essential condition.

Occlusion of the artery may however alter the loudness and quality of the sounds. These effects are best brought out by lowering the compressing pressure in steps and noting at each step the intensity and character of the sounds, first while the artery is open, and then while it is occluded. The artery in such experiments must be so occluded as not to alter its position in the stethoscope or arteriograph, and precautions must be taken to avoid such effects upon the quality and loudness of the sounds as might result from variations in the size of the cleft where the artery passes through the aperture of the stethoscope. The former requirement is met by employing the well-known method of looping a thread around the artery and occluding the artery by drawing the ends of the thread through a glass tube held rigidly in place and so that its orifice just touches the artery. And the latter requirement is met either by filling the cleft with vaseline or by leaving open the side tube of the stethoscope. It might be added, however, that excepting some loss of sound the condition of the side tube, whether open or closed, has been without material effect.

The results obtained in four typical experiments are here given in tabular form (Table III). It will be seen that the changes wrought in the sounds by occlusion of the artery are by no means constant. It is nevertheless possible to recognize certain more or less constant effects:

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TABLE III

Showing the modification of sound by occlusion

PHASE	EXPERIMENT 7	EXPERIMENT 9	EXPERIMENT 16	EXPERIMENT 17	EXPERIMENT 20
Early first	Slightly louder, duller	Disappears	No change, or increases first and then disappears	Fainter and disappears	Duller and very faint
Late first	Fainter and duller		Duller, lower pitch, slightly fainter, or slightly higher pitch, then fainter		Fainter
Second	Fainter and duller	Feebler and duller	Same as late first	Murmur disappears, sounds become very faint and duller; later not so faint	Duller and fainter, murmur disappears
Early third	No change in intensity, duller		Same as late first	Remain loud but become dull	Duller and fainter
Late third	Duller and slightly lower pitch, intensity same or louder	Snapping quality almost disappears, intensity diminishes	Same as late first	Little change in intensity or quality	Changed but slightly, perhaps slightly higher
Early fourth	Louder and duller	Duller, no change in intensity	Clearer, slightly higher, no change in intensity		Very slight change
Late fourth	Slightly louder and more pistol-shot	No change in intensity, higher in pitch	Higher pitch and slightly louder	Slightly louder and higher	Very slight change
No compression	Louder and duller	Louder, no change in character			Very slight change

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Early first phase: The sounds become fainter and disappear; occasionally they first increase in intensity and then become fainter.

Late first phase: The sounds become fainter.

Second phase: The murmur disappears and the sounds become fainter.

Early third phase: The sounds either become slightly fainter or suffer no change in intensity.

Late third phase: Changes in intensity, if present, are slight, and then it either increases or decreases.

Early fourth phase: The sounds either do not change or increase slightly in intensity.

Late fourth phase: The intensity of the sounds is usually increased.

Zero compression: The intensity of sounds is always increased.

In an effort to ascertain the factors actually at work in producing these alterations in the intensity of the sounds we will analyze a record of the pressure changes in the compression chamber obtained in one of these occlusion experiments. It is seen in Table IV that during the first and early second phases occlusion elevates the base line (slight rise of compressing pressure), reduces the amplitude of the oscillations (extension upward of the lower cone and rise of compressing pressure), and increases the amplitude of the peripheral pulse (reflection of the pulse wave). At this stage of the experiment undoubtedly only a very small quantity of blood succeeds in traversing the compression chamber with each pulse. The impact therefore could not extend very far from the point where it strikes; and as the artery fills, the point of impact would be carried away from the stethoscope. This and the enfeeblement of sound that would be associated with a rise of the compressing pressure, might suffice to account for the diminution in the intensity of the sounds usually noted. At the same time it is conceivable that occlusion of the artery might increase perceptibly the force of water hammer and might facilitate the transmission of the impact that causes the sound (see below). Both of these factors might act to increase the intensity of the sounds, at least until the point of their origin had receded some distance from the stethoscope. Under these circumstances the sounds might at first increase in intensity and then decrease, as actually happens in a certain number of the cases (Table III, experiment 16).

In the late second phase occlusion for the first time alters materially the configuration of the compression pulse. The alterations are as follows (See Table IV):

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TABLE IV

Analysis of a record (Experimental 17) showing the effect of occluding the artery peripherally.

PHASE	ELEVATION OF BASE LINE UPON OCCLUSION	AMPLITUDE FIRST CREST				TIME TO FIRST CREST		TOTAL AMPLITUDE				TIME ELAPSING BETWEEN FIRST AND SECOND CRESTS		AMPLITUDE FIRST TO SECOND CREST		SOUND CHANGES
		Open		Closed		Open	Closed	Open		Closed		Open	Closed	Open	Closed	
		Ave.	Range	Ave.	Range			Ave.	Range	Ave.	Range					
	mm.	mm.	mm.	mm.	mm.	sec.	sec.	mm.	mm.	mm.	mm.	sec.	sec.	mm.	mm.	
First	3−							8.1	7.0- 9.0	7.8	6.7- 9.0					Fainter
Early second	3−							11.5	10 8–12 0	10.9	10.0–11.5					Fainter
Late second	3−	12 1	12 0–13 5	9 8	9 5–10 0	0.042–0 05	0.033–0 042	14.5	14.2–14.8	14.8	14.5–15.0			2.4*	5.0*	Remain fairly loud
Third	3+	12 1	11 5–12 5	10 6	10 0–11 2	0 03	0.03	15 0	14.0–15 5	15 2	14.5–16.0	0 050	0 022	3.1	4.6	Remain loud
Last third to early fourth	3+ +	8 6	8 0–9 7	6 9	5 5–8 5	0 03	0 024–0 023	11.6	11.0–12 0	11.1	10 0–12.7	0.072	0.022	3.0	4.2	Little change in intensity or quality
Fourth	1	4 8	4 4–5.0	5 0	4 8–5 2	0 02	0 02–0.022	7.5	7 0–8.0	8.6	8 0- 9.0	0.065	0.015	2.7	3.6	Slightly louder

* First crest not very clear.

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(a) The amplitude of the first crest is lowered from 12.2 to 9.8 mm. (b) The time to the first crest is reduced from about 0.043 to 0.037 second. (c) The amplitude subtended by the first and second crests is increased from 2.4 to 5.0 mm. (d) The total amplitude is increased very slightly—from 14.5 to 14.8 mm. If we take the ratio of the amplitude of the first crest to the time it is attained as a rough index to water hammer action, we have evidence in the foregoing figures that occlusion reduces slightly the force of the water hammer. We can understand, therefore, why, upon occlusion, the sounds, though fainter, "remain fairly loud."

While the third phase is at its height the effects of occlusion upon the configuration of the compression pulse are similar to those just described: (a) The amplitude of the first crest is lowered slightly,—from 12.1 to 10.6 mm. (b) The time to the first crest is not perceptibly altered; it is in the vicinity of 0.02 second under both conditions. (c) The amplitude subtended by the first and second crests is increased from 3.1 to 4.6 mm. (d) Now the second crest is attained very much more quickly while the artery is occluded—0.022, as compared with 0.05 second. The second rise, which is due to the accumulation of blood in the occluded artery, is therefore almost as steep as the first. (e) The total amplitude again remains practically unchanged—15 mm. while open and 15.2 mm. while closed. Consequently, for reasons given with the preceding set of conditions, it seems obvious, on the basis of water hammer, that the sounds should "remain loud" when the artery is occluded.

The changes noted upon occluding the artery when the sounds are in the vicinity of the limit between the third and fourth phases are as follows: (a) The amplitude of the first crest is lowered from 8.6 to 6.9 mm. (b) The time to the first crest is reduced from 0.03 to 0.023 second. (c) The amplitude subtended by the first and second crests is increased from 4 to 4.2 mm. (d) The second crest is attained more quickly—0.022 second as compared with 0.072 second. (e) The total amplitude is very slightly reduced—from 11.6 to 11.1 mm. Here the sounds are changed but little in intensity or quality. The figures would seem to indicate that water hammer action is slightly increased; and inasmuch as the sounds are already dull, little change in their quality is to be expected.

When the sounds are well along in the fourth phase the following changes in the configuration of the compression pulse are to be noted: (a) The amplitude of the first crest, which is now not clearly indicated,

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is not especially changed—5 mm. as compared with 4.8 mm. (b) Neither does the time to the first crest change appreciably; (c) but the amplitude subtended by the first and second crests, considering the total amplitude, is decidedly increased—from 2.7 mm. to 3.6 mm.; (d) while the second crest is attained very much more rapidly—0.018 second as compared with 0.065 second; (e) and the increase in total amplitude is now relatively the greatest of the whole series—8.6 from 7.5 mm. It would therefore seem that in this part of the experiment water hammer, as indicated by the amplitude and steepness of the first rise, is not altered.[5] On the other hand, the distention caused by the second rise when the artery is occluded now assumes a position of considerable importance relatively: although it is not quite so high as, it now reaches its crest in less time than, the first rise. Its tendency to set the arterial walls into vibration would therefore be quite as marked as that of the first rise. Inasmuch, therefore, as the first rise is not materially altered by occlusion of the artery whereas the second rise then practically comes into being, it is easy to comprehend why the sounds become "slightly louder upon occlusion."

A hurried analysis of this record therefore shows that the changes in sound caused by the occlusion of the artery in this particular experiment are explicable on the basis of water hammer action when due allowance is made for the effects of such accessory changes as the extension of the lower cone, the distention of the peripheral artery by the pulsatile entrance of the blood, and changes in the compressing pressure. We do not happen to have made any record of an experiment, such as No. 7, Table III, in which occlusion of the artery in the third phase caused the sounds to increase in intensity. It is therefore impossible to determine whether such an increase is due to an increase of water hammer action or of some other factor. We have as yet no explanation to offer with regard to the cause of the dulling of the sounds so often noted in these occlusion experiments.

[5] This, it might be noted, is an interesting confirmation of our assumption that the first crest is the result of water hammer; for at this stage of the experiment there is no reason why occlusion of the artery should alter materially any of the factors that participate in water hammer: the lower artery is already fairly full, the lower cone is practically eliminated, and the compressed segment now alters its capacity only through the filling permitted by the elasticity of the arterial wall.

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Location of the loudest and the characteristic sounds

In the dog the sounds, when loud, can be heard with the phonendoscope over the artery above the compression chamber (of this more later) and over the upper and lower ends of the compression chamber. It is interesting, however, that these sounds usually differ somewhat from those heard over the lower artery in being somewhat blowing, or at least, duller, in character; the characteristic snap usually is first clearly heard at the point where the artery leaves the arteriograph.

The distance along the distal artery the characteristic sounds can be heard with the stethoscope depends somewhat upon the sound phase. In the early first phase the sounds are heard best close to the compression chamber; at a distance of one or two centimeters they usually can no longer be heard. In the second phase the sounds are transmitted with diminishing intensity a considerable distance down the artery, though the murmurish quality may not be heard beyond 4 cm. During the later phases the sounds are very well transmitted; they diminish slightly with the distance, but can be well heard along the whole course of the freed artery. During the first phase, when the sound is clearly audible close to the arteriograph but not at a distance, it is found that upon occluding the artery distally the sound, though fainter, may become almost equally audible along the entire length of the distended artery.

Essentially similar results are obtainable in man when the sounds are examined over the armlet and at different distances below it. In these observations, we have used a Riva Rocci tube 4 cm. wide, because of all of the armlets now in use this is composed of the fewest layers of material; the button of the phonendoscope resting on it is separated from the underlying tissues by two thin layers of cloth-covered rubber only. Sounds can be heard through this armlet along the course of the brachial artery but they are distinctly fainter over the upper than the lower half of the tube (20). In the arm below the tube the sounds are loudest at the lower edge of the band and their intensity diminishes along the course of the artery though they can be heard for a considerable distance (20). It is scarcely possible to duplicate the observations on the effects of occluding the artery because in man, on account of the simultaneous compression of the veins, the arteries are distended to a certain extent in all parts of the observation.

These results leave little room for doubting that the characteristic Korotkoff sounds start at the lower end of the compressed segment of

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artery; that they are transmitted from this point along the course of the artery; and that they are transmitted for a longer distance along a full than along an empty artery. The latter fact furnishes additional evidence for the view that the sound is developed along the artery by the transmission of a wave. This wave would not be well transmitted when it is started through an empty artery by a small volume of blood. Distending the artery would, however, and does, increase its ability to transmit shocks.

Location of sensation

As is well known the subject of a blood pressure estimation experiences in his arm a sensation resembling a rather sharp shock as long as the compressing pressure lies in the systolic-diastolic region. At all other compressing pressures the pulsatile sensation is not that of a shock. Now that evidence has been obtained indicating that the physical basis of the process enacted under the armlet is water hammer, any one who has felt this shock will recognize the resemblance of the sensation to that which one would expect water hammer to exert.

In my own experience the shock has always been most distinctly felt under the lower part of the armlet (20) where the impact of the water hammer must take place. In order, however, to test this matter in a wholly unbiased way, we have had several good subjects for this purpose, who were unfamiliar with the object of the tests, point continually with the finger to the spot where the sensation was most distinct while blood pressure estimations were being made on them by the method of continuous escapement. In each case the shock was located under the lower edge of the armlet and in most instances at a point that corresponded with the lower 1 or 2 cm. of the pneumatic bag. In all probability this is about the level at which the artery begins to open out from beneath the compression.

Sounds heard central to the compression

It has been maintained that because in man the Korotkoff sounds cannot be heard above the armlet they therefore cannot be produced by any process taking place under the cuff itself (4). Even if this were true it would not preclude the possibility that sounds originate in a process enacted at the lower edge of the armlet. But as a matter of fact sounds can be heard in the artery above the cuff (21).

We have not investigated this question systematically; indeed ob-

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servations have been made on only two subjects. Both were normal young men with blood pressures that might be regarded as at the upper limit of normal and with normal compression sounds. They were selected for these observations because they had long thin arms. The armlet was first fastened to the arm in the usual position and the artery ausculted with the phonendoscope in the bend of the elbow. Then the armlet was shifted to a lower position and the artery auscultated centrally in its most superficial position between the biceps and triceps muscles. The results obtained in both cases were essentially alike; we will therefore describe only one set.

Listening below the cuff, the first sounds were heard at 128 mm. Hg; they became duller at 90 mm. and disappeared at from 85 to 75 mm. Listening above, no sound was audible when the arm was uncompressed. At 160 mm. Hg, however, that is, well above systolic pressure, a sound could be heard. This sound became perceptibly fainter at 124 mm. Hg and at the same time a second sound following the one first heard became audible. The interval between these two sounds diminished as the compression decreased until at about 90 mm. Hg it was no longer possible to distinguish between them; and soon thereafter no sound could be heard at all. It should be added that the sound audible above the armlet when the artery is occluded by a pressure in excess of the systolic pressure does not owe its origin to any property peculiar to the armlet; for the same sound is obtained when the artery is occluded by the finger.

In the dog it is difficult to find room for the stethoscope above the compression chamber and still have a large enough artery in the arteriograph to yield good sounds. But when the phonendoscope is placed on the distal artery a sound, probably emanating from the uncompressed artery above, can be heard even when the compressing pressure exceeds the systolic pressure. As in the case of man a second sound becomes audible at lower compressing pressures. When first heard this second sound is well separated from the first and has the characteristics of the pistol shot sound. As the compressing pressure falls the second sound goes through the five phases, while the first sound steadily grows fainter and approaches closer and closer to the second, until the two merge.

In both man and the dog the first of the two sounds described above is probably developed or enhanced, as the case may be, by the added energy the pulse delivers to the artery when it is occluded. In man it is picked up directly from the upper artery by the phonendoscope; in

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the dog it is transmitted as sound to the phonendoscope below. On the other hand in man the second sound is transmitted upward perhaps partly as sound, though it is conceivable that it is also developed locally by the retrograde impact of water hammer, while in the dog it is in the main developed locally by the transmitted impact. The fact that they merge as the compressing pressure falls is in keeping with the observation that the Korotkoff sounds under similar circumstances appear earlier and earlier in the pulse cycle.

Behavior of the central and peripheral arterial pressures during decompression

It has been shown that the Korotkoff sounds are a manifestation of water hammer; we should therefore expect the arterial pressures central and peripheral of the compression chamber to manifest the changes characteristic of water hammer.

Peripheral pressure. Presumptive evidence has already been presented indicating the existence of peripheral pressure effects consistent with the premises: while loud sounds are in evidence the initial rise of the peripheral pulse may be higher than the crest proper of the pulse. Direct proof of the existence of the peripheral pressure effects of water hammer has been obtained in an experiment in which the artery was cut below the stethoscope and the central stump connected with a mercury manometer through a stopcock which in one position permitted of the free transmission of the pressure and in another position acted as a maximum valve (22). It should be added that, through an oversight, the valve was not delicate enough to give the best results. The results obtained can therefore be regarded as only qualitatively correct; they, however, suffice for present purposes. The experiment shows conclusively (8 estimations) that while those sounds are audible that are well transmitted along the course of the artery, namely, the third phase sounds and possibly the late second phase sounds also, the maximum pressure is higher by from 8 to 18 mm. Hg than the maximum end pressure of the uncompressed artery. During the fourth phase the maximum pressure is not so high as during the third.

Central pressure. The water hammer effect is produced in the compression chamber by the entrance of blood into the opening artery with a speed that exceeds the normal rate of flow. But blood cannot be furnished from above at a velocity greater than the normal with-

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out causing some fall of pressure centrally. We would therefore expect the central pressures to fall momentarily in each pulse while the water hammer is acting, and since the velocity of flow is the main factor that determines the force of the water hammer, we would further expect this fall of the central pressures to vary with the intensity of the sounds. Whether or not this occurs in animals we have not determined. It, or something analogous to it, does, however, occur under similar conditions in a circulation schema. In this connection we quote from an article published by the author in 1904 (14, p. 75).

. . . . With each diminution of the outside (*compressing*[6]) pressure, with its consequent increase in the velocity of blood flow, the *central* pressure in the schema falls slightly. But we find that as we approach the pressure at which maximum pulsations are obtained, this diminution in the minimum (and maximum) pressure is accelerated,[7] but that the pressure recovers when the amplitude of the *compression* pulsations begins to diminish, and that it then continues to fall off gradually. This accelerated diminution of the minimum (and maximum) pressure is quite independent of variations in the rate of *peripheral* flow and has not as yet received a satisfactory explanation.

This result, then impossible of explanation, now is readily accounted for. Indeed it is a necessary manifestation of water hammer as produced by the methods employed in the estimation of blood pressure. It will be noted that it manifests itself in the circulation schema when the compressing pressures lie between those that determine the critical increase and decrease in the amplitude of compression oscillations, which mark respectively the systolic and the diastolic pressures.

SUMMARY

The more important observations and conclusions of this investigation may be summarized as follows:

The Korotkoff sounds are produced by water hammer.

1. The evidence in favor of this conclusion is as follows:

a. While sounds are in evidence, blood enters the compressed artery with a velocity far in excess of the normal.

b. When the sounds are loud (third phase) the artery in the compression chamber can be made to act as a hydraulic ram through the peripheral artery.

[6] Italicized words are added in order to make the context clear.

[7] As a matter of fact the acceleration begins during decompression with the first abrupt increase in amplitude of the compression pulse (see Table III and fig. 12 of the article referred to (14) for further details).

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c. The pressures central of the compressed artery (circulation schema) fall while the wider compression oscillations are recording.

d. The configuration of the compression pulse conforms with the requirements of the water hammer hypothesis.

e. The sounds are located in that phase of the pulse cycle in which a water hammer would strike.

f. The intensity of the sounds varies during decompression as the values, obtained from records, assumed to indicate the force of the water hammer.

g. Calculation locates the initial site of sound production in the lower end of the compression chamber.

h. That the sound is produced by a sudden impact is indicated by the fact that the first of the series of vibrations associated with each sound is the highest.

i. The form of the pulse beyond the compression chamber is such, in certain stages at least, as could be produced only by a sudden impact more forcible than any the pulse itself could strike.

j. The sounds are loudest at the lower edge of the compression chamber.

k. The sensation perceived in the arm during the systolic-diastolic phase of decompression is localized where water hammer would strike and has the characteristics of a blow delivered by water hammer.

2. The main objections to other views on the origin of the Korotkoff sounds are as follows:

a. The fact that the Korotkoff sounds can still be obtained when the artery is occluded at the lower orifice of the compression chamber and when the artery below the compression chamber is distended with blood renders untenable views based on the presence of an empty peripheral artery (3).

b. Views assigning prime significance to the tissues surrounding the artery (6) are invalidated by the fact that the bare artery suffices for the production of characteristic sounds.

c. Resonance of the air chamber (8) is shown to be unessential for the production of characteristic and loud sounds. The evidence presented in support of this view is shown to be open to a wholly different interpretation, namely, limitation of the movements of the walls of the compressed artery.

d. The view ascribing the sounds solely to changes in the form of the tube in the compression chamber (5) is based upon a wrong conception of the sequence of these changes in form. Furthermore, it is shown

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that the artery below the compression chamber during certain of the sound phases contributes to sound production.

3. The mechanism of sound production, in a word, is that the water hammer moving through the artery in the compression chamber, under usual circumstances, strikes the stagnant blood in the uncompressed artery below and distends the artery there so as to give rise to sound. The wave started by this impact is transmitted down (and up?) the artery with sufficient amplitude to produce sound locally as it proceeds, but only when the volume of blood coming through is sufficient and when the lower artery already is fairly full of blood, and therefore ordinarily only in the late second and third sound phases.

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PROCEEDINGS OF THE AMERICAN PHYSIOLOGICAL SOCIETY

TWENTY-EIGHTH ANNUAL MEETING

Boston, December 27, 28 and 29, 1915

Food accessories. T. B. OSBORNE AND L. B. MENDEL.

Food accessories. E. V. MCCOLLUM.

Food accessories. CARL VOEGTLIN.

The formation and structure of the fibringel. W. H. HOWELL.

Experiments on the mechanism of osmosis. JACQUES LOEB.

Further observations on over-activity of the cervical sympathetic. W. B. CANNON and REGINALD FITZ.

In an animal with rapid heart, falling hair, increased excitability, and a steadily mounting metabolism, which had reached about 60 per cent above the average—all these symptoms resulting from union of the phrenic nerve to the cervical sympathetic trunk—removal of the thyroid gland on the operated side stopped the progress of the disease and brought the metabolism down within normal limits. Whereas other animals with the disease had died within three months of the first appearance of the symptoms, this animal lived normally for seven months after the operation, and was then purposely killed.

Some new observations on the uric acid content of the blood. OTTO FOLIN and R. D. BELL (by invitation), with the assistance of G. LE B. FOSTER.

On continuous insufflation through the humerus in fowls. A. L. MEYER and S. J. MELTZER.

In fowls the bones are connected with the air sacs and lungs. In our experiments a continuous current of air was insufflated through the humeri in chickens; in most experiments the air escaped through a tracheal cannula. The continuous insufflation was made under various air pressures and the duration varied from a few seconds to more than two hours. The insufflation was made either with pure air or air admixed with ether or with carbon dioxide. We shall report here only a few of the facts which were observed in these experiments.

When the insufflation pressure was about 10 mm. of mercury, all respiratory oscillations disappeared; the thorax stood still in a more

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or less exaggerated state of inspiration. The degree of pressure, required to produce such a stand-still apparently varied with the size and perhaps also with the vigor of the animal. Fifteen millimeters never failed to produce a stand-still. On the interruption of the insufflation in most of the experiments the thorax immediately assumed an expiratory state in which it continued to stand still variously from a few seconds to half a minute and longer. Following this, inspiratory oscillations set in which very gradually resumed their former depth. Ether invariably prolonged the state of the expiratory after-effect. In a few instances, a stand-still in a reduced inspiratory state preceded the expiratory after-effect. The inspiratory stand-still during insufflation was soon interrupted by some moderate oscillations, when CO_2 was added to the insufflated air. Insufflation of air containing 3 per cent of CO_2 or more does not bring on a stand-still; on the contrary, it increases the inspiratory and expiratory amplitudes of the respiration.

The prolonged continuous insufflation, practiced in these animals, is surely capable of removing a good deal of CO_2 from the body, much more so than in any form of rhythmical artificial respiration or in forced respiration. Nevertheless, in no instance did a symptom make its appearance during or after the insufflation which could be interpreted as "shock." Furthermore, the line indicating the expiratory stand-still ran along the peaks of the expiratory oscillations, and some times even above them. In fowls the expiration is even normally of an active character. The apnoea, then, which follows prolonged insufflation means a tetanic contraction of the expiratory muscles. Our experiments, therefore, demonstrate the fact that the apnoea vera which follows continuous insufflation does not consist in a passive state of the thorax, but rather in the production of a tetanic state of the respiratory muscles, chiefly those of expiration. Since the continuous insufflation in our experiments means a considerable reduction in the content of CO_2 of the body, or as it is now called, acapnia, it follows that in our experiments the acapnia had a stimulating effect upon the respiratory musles and not as it is usually assumed that the lowering of the CO_2 means the reduction or abolition of a stimulus. On the other hand, the experiments in which CO_2 was added to the insufflated air, the respiratory rhythm was started or the unsuppressed rhythmical oscillations were augmented. In other words, our experiments seem to show that the reduction of CO_2 acts as a stimulus apt to produce a tetanus of the respiratory muscles, while the increase of CO_2 favors the augmentation of the respiratory rhythm.

The influence of the adrenals on the kidneys. E. K. MARSHALL and D. M. DAVIS (by invitation).

Heredity and internal secretion in the origin of cancer in mice. LEO LOEB.

Different strains of mice kept in the same environment differ very much in the frequency with which cancer occurs among them. In succeeding generations the percentage figure for cancer is fairly constant

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in different strains of mice. Equally characteristic for different strains is the age at which cancer occurs. Hybridization experiments confirm and extend these conclusions. In our experiments the tendency to cancer was not in the majority of cases a recessive character.

These data are a prerequisite for further studies of factors responsible for the spontaneous development of cancer as well as for attempts to find a rational basis for diminishing the frequency of cancer.

In former investigations we found that a combination of a mechanical stimulus and the influence of a substance secreted by the corpus luteum led to the production of rapidly growing tumor like newformations, the deciduomata. These facts as well as the significance of the corpus luteum for the growth of the mammary gland suggested a possible importance of the corpus luteum for the spontaneous development of cancer in mice. We found that castration of mice at or below the age of six months (corresponding to a period of life, when the animals are already sexually mature) diminished the cancer incidence in a very pronounced way: the cancer rate fell from 60 or 70 per cent in normal mice to 9 per cent in castrated mice.

Non-breeding mice with functioning ovaries develop cancer in a somewhat smaller percentage of cases and at a somewhat higher age than normal breeding mice. The influence of prevention of breeding is much less marked than castration, a finding in accordance with the fact that, while castration eliminates the effect of the corpora lutea, non-breeding merely diminishes or modifies it. Non-breeding may also diminish mechanical irritation of the mammary glands. It appears probable that with the coöperation of hereditary factors all those internal secretions are factors in the origin of cancer which initiate or sustain continuous or periodic growth processes.

The effect of X-rays on cancer immunity. JAMES B. MURPHY.

The presence of posterior lobe secretion in the cerebro-spinal fluid. HARVEY CUSHING and GILBERT HORRAX (by invitation).

The influence of gastrectomy on subsequent pancreatectomy in dogs. J. R. MURLIN and J. E. SWEET.

In 1913 Murlin and Kramer[1] reported the observations that sodium carbonate reduces the output of sugar in the urine of depancreatized dogs and hydrochloric acid increases it. Since the pancreas produces sodium carbonate in proportion to the hydrochloric acid produced by the stomach (Pawlow, Cohnheim and Klee) these observations suggested the possibility that the consequences of pancreatectomy may be due in part to the unneutralized HCl of the stomach. This view was presented in brief in November 1913.[2] Two dogs, in which the stomach contents were excluded from the intestine by a band placed

[1] Journal of Biological Chemistry, 1913, xv, 365.

[2] Murlin: Postgraduate, N. Y., 1913, November number.

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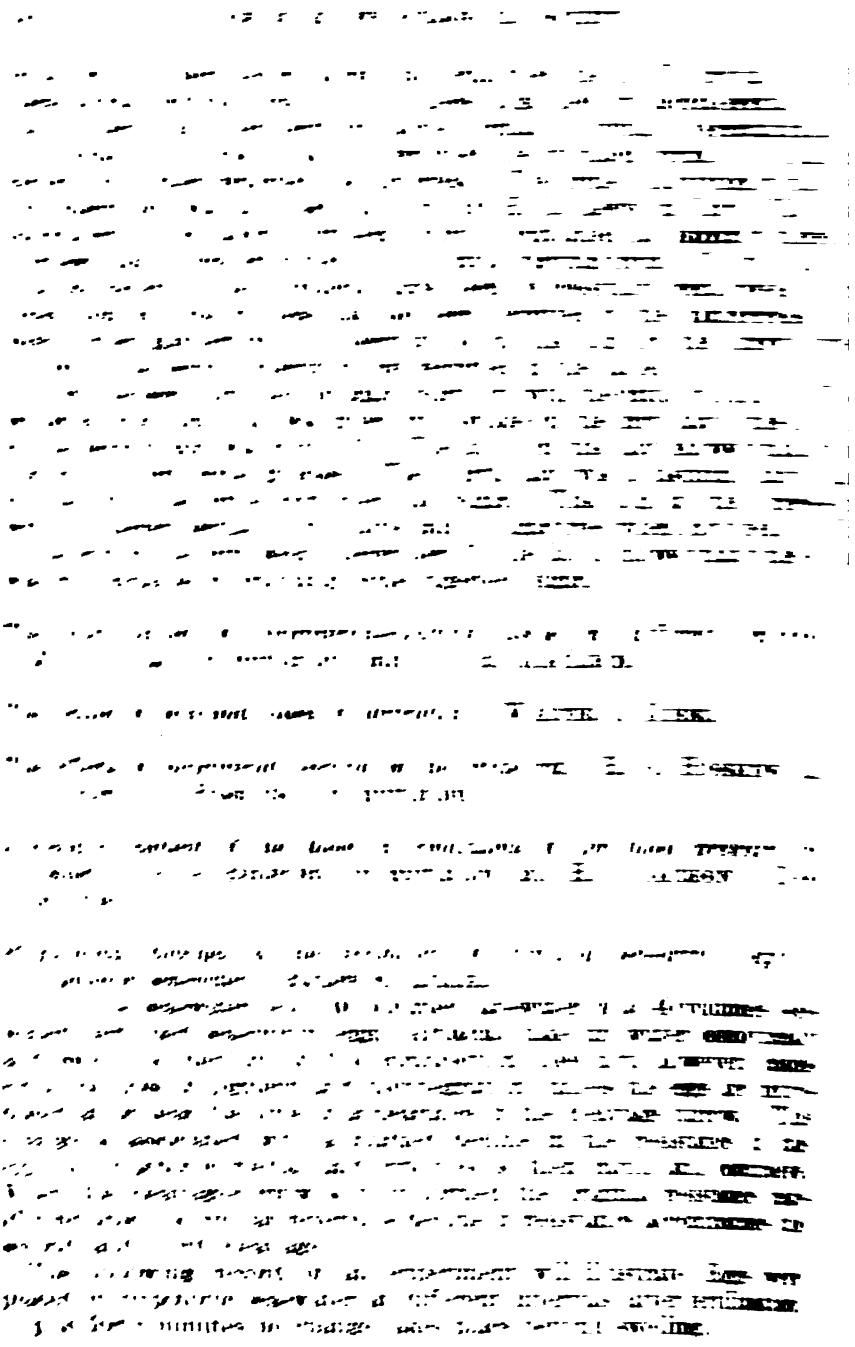
about the pylorus at the time of pancreatectomy, developed no glycosuria for twenty-four hours. Dr. Kramer assisted with these operations.

A third dog whose stomach was removed in the same operation as the pancreas, by Dr. J. A. Hartwell, developed no glycosuria for thirty-six hours and then for two more days showed a D:N ratio below 1. A fourth dog, also operated by Dr. Hartwell, the pancreas being removed one week after the stomach was removed, gave no sugar in the

Dog VIII. Gastrectomy November 11; Pancreatectomy, December 9, 1915

WEIGHT	DATE	TIME	TOTAL D	URINE TOTAL N	D:N	CO_2	O_2	R. Q.	FOOD AND REMARKS
						L. per hr.	*L. per hr.*		
7.05	Dec. 10	9.10 *a.m.*	2.50	2.16	1.11				Given milk by mistake
6.90	11	9.35	0.305	0.496	0.65				
		10.17–11.17				2.434	3.453	0.73	
		11.17–12.17				2.511	3.331	0.75	
		12.35	0.244	0.40	0.61				50 gm. glucose by tube
									20 gm. glucose subcutaneous
		3.45– 4.46				3.120	4.030	0.77	
		5.15– 6.15				3.039	4.000	0.76	
		6.15– 7.15				3.149	4.316	0.73	
	12								200 cc. milk
6.05	13	3.42– 4.42				2.817	3.921	0.72	No food
		4.42– 5.42				3.596	4.948	0.73	
6.05	14	11.15	0.0						
6.05	15	4.35	0.0						
		4.58– 5.58				2.413	3.408	0.71	
		5.58– 6.58				2.465	3.427	0.72	8.00 *p.m.* fed 30 grams starch c̄ digestive powder
		7.35	0.0	0.378					
6.15	16	9.00 *a.m*	2.84						
		12.20	0.0						1.10 *p.m.* fed 50 grams cracker meal c̄ dog's pancreas
		3.25– 4.25				3.654	4.994	0.73	
		4.25– 5.25				2.873	3.998	0.72	

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TIME AFTER FERTILIZATION AND CONDITION OF EGGS WHEN PLACED IN SOLUTION	PROPORTION OF EGGS CYTOLYZED AFTER 30 M. IN DILUTE SEA-WATER
40 m.	Nearly all eggs intact; 1–2 per cent cytolyzed
42 m.	4–5 per cent cytolyzed
44 m.	*ca.* 10 per cent cytolyzed
46 m. (no furrow visible)	*ca.* one-third (35–45 per cent)
48 m. (furrow beginning in a few eggs)	80–85 per cent cytolyzed
50 m. (furrow in *ca.* half the eggs)	90 per cent or more cytolyzed
52 m. (nearly all eggs cleaving)	95 per cent or more cytolyzed
54 m. (most eggs in 2-cell)	60–70 per cent cytolyzed
56 m. (cleavage complete in nearly all)	25–35 per cent cytolyzed
58 m. (all in 2-cell stage)	*ca.* 20 per cent cytolyzed
60 m. (all in 2-cell stage)	*ca.* 10–15 per cent cytolyzed
62 m. (all in 2-cell stage)	Few cytolyzed (*ca.* 5 per cent)

The minimum of resistance is found *during the formation of the furrow*. Both the decline and the return of resistance are rapid, the greater part of each phase occupying four or five minutes. Evidently the plasma-membrane becomes much less resistant to disruption at the time of cleavage; *i.e.*, its coherence or extensibility is decreased; with this change is probably associated an increase of permeability and a decrease of electrical polarization. A definite change in the physical properties of the membrane is thus associated with division of the cell-body; increased surface-tension, resulting from decreased electrical polarization, is probably the chief factor conditioning the change of form.

Mass-action in the activation effect of butyric acid on unfertilized starfish eggs. RALPH S. LILLIE.

Simple exposure to solutions of butyric acid (in sea-water or van't Hoff's solution) is sufficient to induce complete activation in unfertilized starfish eggs, provided the proper time of exposure is employed. With too brief exposures activation is partial (membrane-formation followed by breakdown in early development); over-exposure injures the eggs and prevents development. The activation of under-exposed eggs may be completed by a second exposure to butyric acid solution, as well as by hypertonic sea-water, cyanide, or high temperature (32°).

The optimum duration of exposure (with which 90 per cent or more eggs form larvæ) varies inversely with the concentration of butyric acid. The following table gives the optimum exposures for solutions of butyric acid in van't Hoff's solution (found in a series of experiments in the second week of June).

The approximate constancy of the product of concentration and time of exposure indicates that the butyric acid activates the egg by combining chemically with some egg-constituent. The time required for the production of a definite quantity of reaction-product (the critical

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CONCENTRATION OF BUTYRIC ACID (C)	OPTIMUM TIME OF EXPOSURE (T)	PRODUCT (C × T × 1000)
0.00075 n.	*ca.* 42 m.	31.5
0.001 n.	*ca.* 35 m.	35
0.0015 n.	20–25 m.	30–37.5 (av. 34)
0.002 n.	*ca.* 15 m.	30
0.0025 n.	12–14 m.	30–35 (av. 32.5)
0.003 n.	8–12 m.	24–36 (av. 30)
0.004 n.	6– 7 m.	24–28 (av. 26)

quantity required for complete activation) should, according to the mass-action law, be inversely proportional to the concentration of the butyric acid (since the concentration of the egg-constituent is to be regarded as constant). The nature of the compound formed is problematical. A typical base like ammonia has no effect in activating the starfish egg. A chemical combination in which an acid takes part is thus indicated as the first stage in the activation of this egg.

The permeability of animal and plant cells. W. J. V. OSTERHOUT.

On the rôle played by electrolytes in determining the permeability of protoplasm. G. H. A. CLOWES.

Three types of muscular response in sea-anemones. G. H. PARKER.

The relation of certain muscles to oxygen. F. S. LEE, A. E. GUENTHER and H. E. MELENEY (by invitation).

Influences affecting voluntary muscular work—especially age and tobacco. WARREN P. LOMBARD.

The following is a contribution to individual physiology. The subject has always had excellent health, and has led the regular life of a teacher. In December and the following spring of 1890, when thirty-five years old, he made a careful study of the influences which affected his endurance for voluntary muscular work. In the late summer of 1915, when he was sixty years old, he repeated the experiments, using a duplicate of the old apparatus, and the same methods of work. The flexor muscles of the second finger raised a weight every two seconds, always as high as possible, and this was continued until the greatest possible effort failed to raise the weight. The work was repeated at two hour intervals eight times a day, and during a few days throughout the twenty-four hours. The height to which the weight was lifted by the separate contractions was recorded, and the total amount that the weight was lifted was read off from a work adder.

The experiments showed that his endurance in 1915 was affected by the same influences as in 1890; it was increased by the rest of a night, a meal, and rising atmospheric pressure; and decreased by general fatigue, hunger, falling atmospheric pressure, and smoking; moreover, his diurnal curve of endurance was the same, it being greater at nine to ten o'clock in the morning and nine to ten o'clock at night, and less at

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four to five o'clock in the afternoon and four to five o'clock in the morning. In spite of the fact that he was twenty-five years older, and had done no special work with the muscles in question, his capacity to increase his endurance by training was greater in 1915 than in 1890. In his case, the central nervous mechanisms engaged in the work appear to give out before the peripheral nervous mechanism and the muscles, and it is probable that it is the central nervous system which is chiefly affected by the influences which determine the amount of work that he can do at a given time. If this be true, the greater capacity to increase his endurance in 1915 as compared with 1890, may be the result of the continuous training to which his nervous system has been subjected during the past twenty-five years.

For a number of years preceding 1890 he had the habit of smoking four to five cigars a day, and the number had increased to six or seven a day in 1915. Smoking was found to decrease the endurance in 1915 as in 1890; this effect did not prevent an increase of endurance as a result of training, even when six or seven large cigars were smoked daily; the increase was not as rapid, however, as when no cigars were smoked. Both the dropping of smoking and the resuming of the habit resulted in a temporary lessening of the endurance.

The function of the kidney when deprived of its nerves. WM. C. QUINBY.

Electrocardiographic studies in normal infants. EDWARD B. KRUMBHAAR.

To determine the normal electrocardiogram at different periods of infancy and childhood, records have been taken on 42 subjects from the ninth month of fetal life to twelve years of age. It was found that the fetal heart causes a simple upright monophasic curve. Records made before and after cutting the umbilical cord suggest that the functional capacity of the infant's heart is depressed by this procedure. At birth a right ventricular preponderance is present; the different features of which disappear with considerable constancy by the second or third month. By the sixth month, the infant's electrocardiogram is practically the same as the adult's, except that Q_2 and Q_3 are apt to remain unduly prominent beyond this period. The P–R interval is shorter and sinus arrhythmia is practically absent in the first year. Sinus arrhythmia increases in frequency from the sixth to the twelfth year.

The time relations of auricular systole. CARL J. WIGGERS.

Simultaneous tracings of auricular myograms and intra-auricular pressure curves, were recorded by optical systems. The records presented led to the following conclusions:

1. A short interval after excitation each unit of cardiac tissue begins to contract and continues in this condition on an average, for 0.047 second.

2. When the approximation of two points on the auricle is recorded accurately, the myogram shows three phases: (a) when the contraction of each cardiac unit progresses from one lever attachment to the other. (b) when all auricular tissue is contracting and (c) when contraction of

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one portion goes on at the same time with relaxation of another. This total interval lasting on an average 0.077 second may be designated the *mechanical contraction*.

3. As the rise of intra-auricular pressure precedes *mechanical contraction* of the auricle, on an average, by 0.019 second this interval should be added to the period of the mechanical contraction in order to estimate the entire *systole* of the auricle, which equals on an average 0.11 second.

4. The auricle increases tension within its cavity and that of the ventricle *only* during the early half of its systolic period. The *dynamic systole*, as this portion of the curve is termed, averages 0.0533 second.

5. The interval between the end of auricular systole and beginning of ventricular systole averages 0.0165 second making the $A_s V_s$ interval 0.132 second.

The movements of the mitral valves in relation to auricular and ventricular systoles. A. L. DEAN (by invitation).

Further researches on the relation of the chromotropic action of the vagus to the nodal tissues. H. STEENBOCK, J. A. E. EYSTER and WALTER J. MEEK.

The tension of carbon dioxide and oxygen in the venous blood at rest and at work. WALTER M. BOOTHBY and IRENE SANDIFORD.

If the circulation rate and the arterial (alveolar) carbon dioxide and oxygen tensions are known it is possible, as is shown in our previous paper,[1] to calculate the tension of carbon dioxide and percentage saturation of the haemoglobin in the venous blood.

The carbon dioxide tension can be calculated as in curve VIII, figure IV, making allowance for the effect of partial desaturation of the haemoglobin on the dissociation curve of carbon dioxide or it may be plotted without this correction as though the haemoglobin remained saturated.[1]

This uncorrected venous carbon dioxide tension calculated from the same data as the other curves in figure IV is as follows:

OXYGEN CONSUMPTION	UNCORRECTED VENOUS CO_2 TENSION
cc.	mm.
175	47.8
200	48.5
300	51.9
400	54.5
500	56.5
600	58.7
700	60.3
800	61.3
1000	64.0

[1] Boothby: A determination of the circulation rate in man at rest and at work 1915, Amer. Jour. Physiol., xxxvii, 2.

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Since the publication of the above paper we have performed two hundred and forty-four experiments on the direct determination of the uncorrected venous carbon dioxide and oxygen tensions according to the recent method described by Christiansen, Douglas, and Haldane.[1]

We have averaged these new experiments in the same manner as those in the previous paper and the results are given in the following table:

OXYGEN CONSUMPTION	UNCORRECTED VENOUS CO_2 TENSION
cc.	mm.
225	51.5
473	57.0
600	60.0
708	62.4
805	63.3

The points as determined fall on a line parallel to but from 1 to 2 mm. higher than the curve calculated from the data of the previous experiments.

This discrepancy, while slight, is consistent with the constant errors existing in the two methods of experimentation. In both methods the assumption is made that none of the blood makes a complete circuit during the time of the experiment; in the first set the error from this assumption will cause the calculated tension to be slightly too low; in the present series the same constant error will cause the tension determined experimentally to be correspondingly too high.

The points for the percentage saturation of the haemoglobin are fewer in number and do not correspond to the calculated curve as well as in the case of the carbon dioxide tension. The technic is more difficult with oxygen, especially at work, and the inhalation of pure nitrogen makes the experiments somewhat dangerous so that their number was limited.

The following table gives the percentage saturation of the haemoglobin determined experimentally for various oxygen consumptions:

OXYGEN CONSUMPTION	PERCENTAGE SATURATION HAEMOGLOBIN
cc.	mm.
225	69.5
603	59.0
704	53.1

The chief physical mechanisms concerned in clinical methods of measuring blood-pressure.[2] CLYDE BROOKS and A. B. LUCKHARDT.

[1] Christiansen, Douglas, and Haldane: The absorption and dissociation of carbon dioxide by human blood. 1914, Jour. Physiol., xlviii, 4.

[2] Paper published in full elsewhere in this number.

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Haemodynamical studies. R. BURTON-OPITZ. Read by title.

The mechanism of the arterial compression sounds of Korotkoff. JOSEPH ERLANGER.

The responses of the vasomotor mechanism to different rates of stimulation. CHARLES M. GRUBER.

The response of the vasomotor mechanism is affected by different rates as well as by different strengths of stimuli. A long series of experiments upon cats under urethane anaesthesia showed that slow rates 4 to 6 per second are favorable in bringing about a reflex fall in blood pressure and a rapid rate 20 per second is favorable in bringing about a rise in blood pressure, when the current is weak (5.8 to 17Z units). When the current is strong, 494Z units, a slower rate 1 per two seconds or 1 per second interruption is favorable in producing reflex vasodilation.

Vasomotor summations. E. G. MARTIN and P. G. STILES.

Blood changes following hemorrhage and perfusion. THEODORE HOUGH and J. A. WADDELL.

Five dogs and three rabbits bled from artery or vein and immediately perfused with 0.9 per cent saline or with 0.9 per cent saline and 2.5 per cent gelatin. Counts of reds and whites, and hemoglobin determinations; in one animal daily duplicate determinations of each of these for a period of eight weeks. (1) The post-hemorrhagic fall of hemoglobin and reds, noted by previous observers, occurred generally with saline perfusions, but was absent in four out of the five cases of gelatin-saline perfusion. (2) When regeneration began to show its effect two to four days after hemorrhage, in all animals but one the color index (ratio of hemoglobin to erythrocytes) rose and for at least two or three weeks remained above normal. This indicates that the newly formed corpuscle contains more hemoglobin than those that have been longer in the circulation; that is to say, erythrocytes may and generally do undergo a gradual loss of hemoglobin. (3) The discharge of new erythrocytes on the blood is not a steady process but shows a distinct tendency, in the rabbit at least, to be intermittent. Periods of increase for one or more days were followed by a constant count or even by a fall for several days; then would come another rise. (4) Each rise in the erythrocyte count is accompanied by a distinct rise in the leucocyte count either on the same or the preceding day. It is believed that, in general, when other causes of leucocytosis are controlled or absent, the leucocyte count may be taken as an indication of the degree of activity of the blood forming organs. (5) Periods, lasting from two to six days, of apparent instability of the erythrocytes were observed from time to time. This showed itself in the number of ghosts or the tendency of the erythrocytes to fragment and go to pieces after being mounted on the counting slide. There were indications

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that the older rather than the newly formed corpuscles were affected by this hemolytic action. (6) The tendency of the color index to rise during the first period of regeneration may give place later to a fall which results in its remaining below normal for weeks, despite frequent periods of hematopoiesis. It is suggestsd that the manufacture of hemoglobin calls for an unusual supply of certain amino-acids or other material which, during the early period of blood regeneration, can be furnished by the organism from the reserve supply of its own tissues; later, when this reserve is exhausted, only a limited amount of hemoglobin may be manufactured from the usual food and we may have, and generally at such times do have, a rise of the red blood count unaccompanied by any corresponding rise of the hemoglobin. In such cases hematopoiesis results in a lower color index, in contrast to what is observed during the first periods of hematopoiesis after the hemorrhage.

Experimental and clinical studies on mental defectives. III. The relation of systolic and diastolic blood pressures and their power of adjustment to body position. AMOS W. PETERS and CAROLINE D. BLACKBURN.

Feeble-minded inmates of an institution and some normal subjects were tested for the efficiency of the splanchnic vasomotor mechanism. The pulse rate and the systolic and diastolic blood-pressures were measured after stability had been established in first lying and then standing positions. It was desired to obtain a quantitative expression for the vasomotor tone of these subjects in terms of Crampton's scale of percentage condition. The expected age differences between children and adults in pulse rate and in systolic and diastolic pressures were observed. After changed posture the average pulse rate was increased by 11. The average systolic pressure increased only 4 mm. but the average diastolic pressure increased 18 mm., or in percentage of standing to lying as 2.4, systolic, to 32.1, diastolic. On Crampton's percentage scale of vasomotor tone the average of all the subjects showed a low result viz., 75 per cent. Their cardio-vascular adaptation is poor corresponding to their defective growth-development. Known irregularities in the auscultation phenomena in children (Katzenberger), vitiate the supposed differentiation by this criterion between the normal and the feeble-minded.

Prolonged uniform intravenous injections (Lantern). R. T. WOODYATT.

The destruction of hormones, pro-enzymes, and enzymes, by ultra-violet radiation. W. E. BURGE.

The hormones used were adrenalin and secretin; the pro-enzymes and enzymes, trypsinogen, trypsin, pepsin, ptyalin, amylopsin, taka diastase and enterokinase. Five cc. of a clear solution of the substance to be exposed were introduced into a circular glass vessel 5 cm. in diameter and 1 cm. deep. This was covered with a quartz plate 2

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mm. thick to prevent evaporation and the vessel was partially immersed in running water beneath a quartz mercury-vapor burner operating at 140 volts, 2.3 amperes and 2400 cp. at a distance of 5 cm. The temperature of none of the solutions rose higher than 30°C. during the exposures.

The exposures were made for different lengths of time and the rate of destruction was found to be directly proportional to the length of exposure. All the substances were found to be destroyed after an exposure of about an hour.

When the vessel containing the substances exposed was covered with a clear piece of plate glass 5 mm. thick instead of the quartz plate none of the substances were affected after many hours' exposure. It had been determined that the glass cover used did not transmit wave lengths shorter than 313 $\mu\mu$ hence these shorter wave lengths were the ones which caused the destruction of the substances exposed. It had also been determined that only wave lengths 302 $\mu\mu$ and 297 $\mu\mu$ in the spectrum of the quartz mercury-vapor burner used were effective in coagulating protein. The assumption might be made that these are the specific wave lengths which cause the destruction of the enzymes, pro-enzymes and hormones.

Initial length, initial tension and tone of auricular muscle in relation to myo- and cardiodynamics. ROBERT GESELL.

Is the contraction of smooth muscle accompanied by heat production? (Second Communication.) C. D. SNYDER.

A suitable smooth-muscle-nerve preparation has been made out of a ring of turtle's stomach with left vagus attached. The ring of muscle is slipped in place over a thermopile of the "Gittersäule" type and suspended in a specially devised moist-chamber.

When the muscle was made to contract by stimulating the nerve the results were of a contradictory nature. At times no heat exchange accompanied or resulted from the contraction; at other times equal tensions apparently were accompanied now by heat absorption now by heat production.

The same preparation, without being subjected to any manipulation, upon direct stimulation gave off heat in a wave of two maxima, as Bernstein has recently reported (Pflüger's Archiv, vol. 159).

The first wave of this heat production is doubtless due to the warming caused by the degraded electricity. The second maximum Bernstein further explains is due to the energy exchanges directly concerned with the muscle contraction. In the present author's experience this becomes doubtful for the reason that the same muscle, when dead and similarly stimulated with the electric current, again gave off heat in a wave of two maxima.

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Lifting and the Valsalva experiment: effect on systolic pressure, heart rate and radial pulse curve in man. With a note on labor pains in a rabbit. PERCY M. DAWSON and PAUL C. HODGES, University of Wisconsin.

The object of this research was to determine some of the immediate effects upon the circulation of "exercises of strain." By the latter are meant those muscular efforts which are performed with the glottis closed and which tend to compress the chest thereby causing a rise in intra-thoracic pressure. The forms of strain studied were the Valsalva experiment and lifting.

The Valsalva experiment consists in forced expiration with the closed glottis. The history of this maneuver is dramatic. It includes the account of the bandit, who committed suicide in the very presence of the unsuspecting Roman consul and thus eluded a cross-examination (Valerius Maximus); of Colonel Townsend, "the man who could die and come to life again" (Cheyne); and of the self-induced syncope of E. F. Weber.

The changes in systolic pressure were determined by the auscultatory method supplemented with the Erlanger sphygmomanometer, the inflation of the cuff being rapidly performed by means of highly compressed air. They consisted in (1) a rise first described by Riegel and Frank, '76, first measured by McCurdy, '02, (180–200 mm. Hg); (2) a fall first observed by Weber, '50. The extent of this fall is such that when the pressure in the cuff of the "Erlanger" is 60 mm. or more., no pulsation of the lever could be obtained by us; (3) a rise first observed by Bruck, '07. The latter states that this may reach 200 mm. but we were unable to obtain a rise above 140 mm. which is no more than that obtained by simply holding the breath for a similar length of time. Following the effort the pressure remains elevated subsiding with the disappearance of dyspnea.

The changes in heart-rate were determined with the string galvanometer. They consisted in (1) a slowing (often absent) occurring as soon as the effort began; (2) a quickening first observed (but not measured) by Riegel and Frank, which reaches its maximum shortly after the cessation of the effort; (3) a great slowing followed by a more or less gradual return to normal. For example normal length of cardiac cycle was 0.68 second, at (1) 0.94 second, (2) 0.46 second, (3) 1.26 second, returning again toward normal 0.88 second. These variations are confined to diastole. The A–V interval is unaffected.

The pulse wave was studied with the Dudgeon sphygmograph (weighed after Lewis, '96). The results obtained confirmed those of Riegel and Frank, whose findings have been adequately explained by themselves and by Hill, Bernard and Sequeira, '97.

In a second series of experiments (not yet quite completed) lifting was substituted for the Valsalva experiment. Up to the present our results are similar.

By chance we obtained mean blood pressure and respiratory records in a rabbit during labor. The latter was induced by stimulation of the

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sciatic under urethane and chloral anesthesia. Accompanying the labor pain there was a rise in pressure and slight slowing of the heart rate (accompanied by cessation of the respiratory movements), followed by a fall in pressure to below normal. The return (rise) to normal occupied about 30 seconds.

Comparative studies in the physiology of the gastric hunger contractions in the amphibia and the reptilia. T. L. PATTERSON.

The comparative studies on the amphibia and the reptilia were made on the bullfrog (Rana catesbiana), and the common snapping turtle (Chelydra serpentina), respectively. The usual operative method of procedure was modified in the case of the bullfrog while the ordinary gastrostomy was performed on the turtles. All the bullfrogs were stomostomized. This simple operation consisted of making a circular opening on one side between the ramus of the inferior maxillary, near the angle, and the anterior cornua of the hyoid bone through the skin, the mylohyoid muscle and the lining membrane of the mouth of sufficient size to admit the balloon and the attached rubber tube which connected with the recording manometer.

The gastric tonus in both the frog and the turtle remained practically constant throughout the experimentation. In the turtle, however, there was a marked increase in the amplitudes of the hunger contractions during prolonged starvation directly proportional to the length of the fast and the same was indicated in the frog. The author[1] in a previous paper has shown in the dog that there is a marked increase in the gastric tonus in prolonged starvation, and that this increase is inversely proportional to the decrease in the amplitudes of the hunger contractions. This is just the reverse of what is found in the case of the frog and the turtle. The gastric hunger contractions in the frog were continuous but the hunger movements of the turtle showed periodicity which is one of the characteristic factors used in differentiating the gastric hunger movements from digestive peristalses in all the higher animals so far experimented upon.

From X-ray studies made on the frog's stomach by means of the bismuth coated balloon, as well as observations made on the excised stomach of the same animal the gastric contractions were found to be peristaltic, the peristaltic waves originating within about 1 cm. of the cardia and advancing rhythmically over the stomach. When water, Na_2CO_3—1 per cent solution and HCl—0.5 per cent solution were introduced directly into the stomach of the frog without coming in contact with the mouth they invariably produced inhibition varying in degree with the stimulating power of the substance introduced, it being most marked in the case of the acid and least in the case of the water. When these same substances were introduced directly into the mouth cavity the inhibitory effects produced were very slight, thus indicating that the cerebral processes were not as highly developed as in the case of the higher animals, since introduction of these substances into the mouths

[1] Patterson: Am. Jour. of Physiol., 1915, xxxvii, 316.

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of higher animals is followed by a marked inhibition. The gastric hunger movements are not affected by the removal of the cerebral hemispheres, the graphic record of the normal and the decerebrate animal remaining practically the same which shows again that the cerebral processes exerts no appreciable influence on the gastro-neuro-muscular apparatus of the frog. Records of the frog shortly after feeding showed but very little change from the stomach of the hungry animal, the only observable variation being perhaps a very slight increase in the rate of the contractions. The introduction of the previous mentioned substances directly into the stomachs of higher animals normally produces gastric hunger inhibition but has scarcely no effect upon digestive peristalis, however, in both the hungry and the filled stomach of the frog similar inhibitory effects were produced by them. Therefore, we are justified it seems to me in drawing the conclusion, that in the frog at least, we have a much simplified gastric mechanism which through the processes of evolution has evolved itself in the higher animals into the gastric digestive peristalses and the gastric hunger contractions, the latter of which perhaps may be described as intensified gastric digestive peristalses.

Localization by faradic stimulation in the floor of the fourth ventricle. F. R. MILLER.

By employing the method of unipolar faradization it is possible to determine the location of a number of functions in the floor of the fourth ventricle.

Sherrington and Miller localized deglutition at the inferior fovea. Since the fasciculus solitarius approaches the surface at this point it appears probable that the swallowing is evoked by the stimulation of afferent fibres of the glossopharyngeal and superior laryngeal nerves contained in the fasciculus. By stimulating the same point a secretion of saliva is elicited from the ipsilateral parotid and submaxillary glands, the flow from the parotid being the greater. These glandular effects are probably produced reflexly by stimulation of afferents in the fasciculus solitarius. Salivary secretion may also be excited at two points near the middle line of the medulla at the level of the striae medullares; the anterior point yields submaxillary the posterior point parotid secretion. These two effects probably depend on the stimulation of efferent fibres to the respective glands. With the aid of unipolar stimulation the centres for cardiac inhibition and for movements of the stomach and small intestine were localized in the dorsal vagus nucleus (ala cinerea). These latter results are in agreement with those which Van Gehuchten and Molhant arrived at by histological methods.

Direct evidence of duodenal regurgitation and its influence upon the chemistry and function of the normal human stomach. WILLIAM H. SPENCER, GEORGE P. MEYER, MARTIN E. REHFUSS, and PHILIP B. HAWK.

The absence of bile in the stomach when certain materials are introduced and its presence with certain other substances renders bile an

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uncertain indicator of regurgitation of duodenal contents. The varied results of investigators on this subject can be attributed to their use of bile for this purpose. The uncertainty of the presence of bile led to our further investigating the theory of Boldyreff as to the self-regulation of the acidity of the contents of the stomach by regurgitation of alkaline duodenal juices.

The quantitative estimation of trypsin by the method of casein digestion, was done upon the samples obtained by fractional analysis of the gastric content. Various materials were introduced into the fasting stomach (previously emptied of residua) of normal healthy men and the trypsin values compared with the reaction of the gastric content at ten minute intervals.

Trypsin proved to be the ideal indicator of duodenal regurgitation and was found to be almost constantly present in the fasting and digesting content of the normal human stomach.

In general, the tryptic values were high in gastric contents of low acidity and of alkaline reaction, and low when the gastric contents were of high acid concentration. A fall in acidity was usually accompanied by a rise in tryptic values. When bile was present the tryptic values of the gastric content usually rose, concomitantly with the color changes, but in a non-bile stimulating diet trypsin was constantly present where no traces of bile were found.

0.5 per cent HCl ingestion was followed by a rapid fall in acidity to about 0.2 per cent HCl acid due to a regurgitation of alkaline duodenal contents, as is indicated by a rise in tryptic values coincident with the fall of the acidity.

Sodium bicarbonate in 0.5 per cent solution is held in the stomach until sufficient HCl is secreted to bring the alkalinity to a point where it is non-irritating to the duodenum. The retention is accompanied by high trypsin values—suggesting regurgitation in response to duodenal irritation. Sodium bicarbonate in 0.1 per cent solution hastens the emptying of the stomach either by increasing the motility of the stomach or opening the pylorus.

Sodium bicarbonate solutions do not inhibit human gastric secretion, but seem to have a direct stimulatory effect in most cases. Free HCl appears unnecessary for the opening of the pylorus, for the stomach sometimes empties while its contents are still alkaline in reaction.

Our work in many ways confirms the theory of Boldyreff. This phenomenon of regurgitation occurs however, not only with high acidity but when the gastric contents are made alkaline in reaction and seems to be a constant accompaniment of normal gastric digestion.

The diuretic action of tissue extracts. FRANK P. KNOWLTON.

The appearance of sugar in the digestive secretions of phlorhizin glycosuria. ROY G. PEARCE.

It is generally held that phlorhizin produces a glycosuria by exerting a specific action on the renal mechanism. In the present research the

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salivary, gastric and pancreatic juices of normal and phlorhizinized animals have been examined with regard to their reducing power. While in normal dogs, whose blood sugar was less than 0.13 per cent no sugar could be demonstrated in the above juices, in the case of phlorhizinized dogs a reducing substance was found practically without exception, in the pancreatic and gastric juice, and often in the saliva. The kidneys are not necessary for the production of the phlorhizin effect, inasmuch as ligation of the renal vessels previous to the administration of phlorhizin does not alter the result. The percentile amount of reducing substance present in the juices was in all cases less than that present in the blood at the time of the collection of the juice.

The results in connection with the discovery of Levene, which has been confirmed by Woodyatt, that the bile of phlorhizinized animals contains dextrose, suggest that phlorhizin does not exert a specific action on the renal cells in the production of phlorhizin glycosuria.

The rapidity with which alcohol and some sugars are available as nutriment. H. L. HIGGINS.

Some results of studies on electrical changes in glands. W. B. CANNON and MCKEEN CATTELL.

We have confirmed the observations of Bayliss and Bradford that an electrical change accompanies secretion by the submaxillary gland though the blood supply is cut off or the flow through the duct is stopped, and that the change is absent when secretion fails, though the conditions of secretion remain—changes in blood vessels and blood flow. We conclude therefore that the electrical change is a manifestation of the secretory process.

The direction of the action current from the submaxillary gland may be reversed although secretion occurs as usual in response to stimulation. Reversal is therefore not a sign of a reversed process in the gland.

Stimulation of the sympathetic cord in the thorax or in the neck causes an action current in the thyroid gland. Its latent period (5 to 7 seconds) is much longer than that of the submaxillary. It occurs after the laryngeal nerves are severed. It does not follow injections of pituitrin or pilocarpine, but is marked after injections of adrenalin. It appears on stimulating the adrenal gland through the splanchnic nerves. It is only slight and temporary after anemia mechanically produced.

The latent period of the action current of the adrenal glands is about fifteen seconds. We have recorded the action current of the pancreas after secretin injections, and a plan for a comprehensive study of the glands of internal secretion is now being carried out.

The action of the depressor nerve on the pupil. JOHN AUER.

Stimulation of the depressor nerve in white rabbits narcotized by the subcutaneous injection of 5–10 mgm. of morphine sulphate per kilo, usually causes a definite diminution in size of the pupil. This

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contraction in typical cases is composed of two stages: a sharp prompt, short initial contraction followed by a slower, gradual contraction. Often only the initial contraction is observed, at other times only the slower, gradual contraction.

The initial contraction, when present, is obtained as soon as the nerve is stimulated, before the blood pressure begins to fall. The slower contraction occurs while the blood pressure is falling, and the iris blanches at the same time.

Stronger stimuli are necessary to cause this contraction of the pupil than suffice to bring on the characteristic drop of blood pressure. A strong fall of blood pressure due to a moderate depressor stimulation does not cause any alteration of the pupil.

Stimulation of one depressor may cause a contraction of the pupil on the opposite side.

This pupillary effect cannot be obtained with the same certainty as the fall in blood pressure. After several successful trials, the pupil usually fails to respond for a while.

The two depressors vary in their pupillary effect; one may yield excellent pupillary contractions, the other one none at all.

The stimuli used were rarely longer than five seconds; the strength 100–150 mm. coil distance (Petzold coil).

Section of the sympathetic nerve, or extirpation of the superior cervical ganglion, the depressor of the same side being stimulated several days later, exerts no appreciable effect on the result. The reflex therefore seems to act on the third nerve chiefly, if not entirely.

In addition to this pupillary effect, depressor stimulation at times causes a short wink or a more or less prolonged retraction of the bulbus.

It must be added that a strong winking (closure of the lids being prevented by a speculum) usually causes a very short sharp contraction of the pupil, the Piltz-Westphal phenomenon. This contraction is, however, more rapid than what has been described as the initial contraction on depressor stimulation; moreover, the initial contraction is frequently obtained without any sign of winking.

In rabbits anaesthetized by ether, or which have been allowed to recover from the ether, the depressor pupil effect was not obtained. An increase of reflex irritability is apparently necessary in order to obtain pupillary contraction on depressor stimulation.

Evidence showing the metaphore to be a disguised type of smooth muscle. RAYMOND SPAETH (by invitation). Read by title.

The voluntary innervation of skeletal muscle. E. G. MARTIN and R. W. LOVETT (by invitation).

Comparison of the chemical changes in the central nervous system in pellagra and in animals on an exclusive vegetable diet. M. L. KOCH (by invitation) and CARL VOEGTLIN.

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A study of a lecithin-glucose preparation. ERNEST L. SCOTT.

Lecithin was emulsified by shaking with water in the usual manner and, after having been well mixed with a sugar solution was evaporated to dryness on a water bath. In this way the alcohol was eliminated from the process though we still have a temperature which it is impossible to consider as occurring in an organism. However, in every case a "compound" was obtained which gave all the characteristic reactions of Bing's preparation, i.e., it was precipitated from an ether solution by the addition of a small amount of alcohol. A substance is obtained which is soluble in ether and benzene and from such solutions glucose may be obtained. This glucose precipitates to some extent from a clear solution which has been allowed to stand for a few days but is apparently quantitatively removed by repeated drying and solution in ether. An emulsion of a neutral fat (peanut oil) in place of lecithin treated in a similar manner failed to give any trace of any of the above reactions. One preparation has been prepared giving all of the above reactions which was dried by vacuum desiccation at room temperature.

The rotation of light by an ether solution of the preparation was compared with that of a similar solution of lecithin, and for the lecithin solution $[\alpha]^{D}_{20}$ was found to vary about 85 while that for the preparation varied about 135.

This preparation was further studied by comparing the freezing point of a benzene solution of it with that of a similar solution of the parent lecithin. The results, however, indicate a reaction between solute and solvent and so are inconclusive. It is hoped that further search will reveal a suitable solvent for use in the freezing or boiling point determination. Freezing point determination of lecithin emulsions containing small amounts of glucose indicate a loss of molecular concentration equivalent to 10 per cent to 60 per cent of the glucose present depending upon its concentration.

All of the added sugar may be recovered from such a preparation by removing the lipoid with colloidal iron and estimating the glucose by reduction. If it is supposed that such a compound exists in the blood this falls in line with our experience in estimating the blood sugar. A few qualitative experiments indicate that all of the sugar may be removed from such a preparation by dialysis.

The results so far obtained warrant us, we believe, in continuing the work and perhaps extending it to cover other physical-chemical properties and to other substances, as cholesterol, with which sugar of the blood and tissue might combine.

Effect of excluding pancreatic juice from the intestine on the absorption of nitrogen and fat. JOSEPH H. PRATT.

Metabolism experiments were conducted on six dogs in which the attempt had been made to exclude all the pancreatic secretion from the intestine. In four of the animals the operation was successful and all

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of these showed a marked disturbance in the absorption of nitrogen and fat. Extreme atrophy of the pancreas developed. One animal was studied over a period of two and a half years. During this time eight metabolism tests were made. The disturbance in absorption persisted. The animal finally died of inanition.

The feeding of fresh pancreas and pancreatic preparations resulted in better assimilation of the food. On an exclusive milk diet the fat was well absorbed. In one of the two animals in which there was some escape of pancreatic juice into the intestine there was a moderate disturbance in absorption. In this case only a portion of the pancreas remained unatrophied. In the other dog the absorption was normal. Here the pancreas was unchanged and the communication between the pancreatic ducts and the duodenum had been re-established. When a bit of pancreas measuring 1 cm. in size was left attached to the open main duct there was only a slight disturbance in absorption. A test made nearly six months after the operation and three weeks before the death of the animal showed that 75 per cent of the nitrogen and 66 per cent of the fat were absorbed. At the autopsy on naked eye examination there was nothing that could be recognized as pancreatic tissue in contact with the duodenum.

None of the animals developed diabetes. The fat in the feces was well split. No abnormality was discovered in the gastric digestion.

The fat of the blood in relation to heat production, narcosis and muscular work. J. R. MURLIN and J. A. RICHE.

The major portion of these observations have been reported in a brief communication to the Society for Experimental Biology and Medicine (*Proceedings*, 1915, xiii, p. 7). To these we wish to add the observation that muscular work alters the percentage of blood fat. Dogs permitted to run in a tread mill for one hour at the rate of about four miles per hour, exhibit within the first half hour, sometimes within fifteen minutes, a fall in the percentage of blood fat. At the end of an hour, however, the percentage invariably rises (in carotid blood) above the normal. At the end of an hour's rest following the run the percentage has returned to or below the level which it had before the run. The fluctuations are greater in fat than in lean dogs.

The fat and lipase content in the blood in relation to fat feeding and to fasting. C. W. GREENE and W. S. SUMMERS.

The discovery of the reversible action of lipase by Castle and Loevenhart gave a key, therefore a new impetus to the investigation of the problems of fat transportation and fat metabolism in the animal body. Among the numerous recent papers on fats very little attention has been given to the synchronous variation of fat and lipase content. This relation we have examined in the blood chiefly of dogs under the conditions of prolonged fasting, and immediately following a fat meal.

The fats have been determined by the nephelometric method of

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Bloor,[1] and the lipase by the method of Loevenhart,[2] using $\frac{N}{10}$ sodium hydrate titration of the butyric acid liberated by the lipase acting for a constant time and temperature.

After feeding. The blood of puppies quickly shows a variation in lipase and fat after a meal of milk and cream. The variation has been studied on sets of puppies of the same litter. When killed at three, five, eight, eleven and fourteen hours after feed the blood shows a curve of sharp increase in fat at the fifth to eighth hour, and a smaller increase to the eleventh or fourteenth hour in comparison with the normal. The blood lipase increases slightly during the interval of absorption. Adult individual dogs put on feed after a prolonged fast show only a slight increase in blood fats, but a very marked increase, double and more, of lipase lasting through two or three days before return to an average level.

During fasting. The blood of fasting puppies killed at successive fasting intervals shows a marked increase in the blood fats amounting in one series to 230 per cent of the normal on the eighth day, and 185 per cent on the ninth or last day of the fast. In the meantime the lipase of this series remains about constant for two days, rapidly falls to 45 per cent of the normal at four to six days, and sharply increases to 125 per cent on the ninth day. The cycle of changes occurs in a shorter time with younger animals. The apparent absolute amount of lipase increases with the age of the animals.

In adult dogs fasted for seventeen to twenty-two days intervals, the striking fact is the uniformity of content of fat in the blood. There is a tendency to lower fat content during twelve to fifteen days with a possible upward tendency after fifteen days, both within the limits of experimental error. The lipase curve is typical and like that of the fasting puppies, viz.,a decrease to half the normal or less in four to five days, a persistent low content to six to eight days, after which there is a very regular and even rise in lipase content to the end of the fast. The lipase level in every test on adult dogs was higher at the end of the fast than at the beginning, a fact that is significant in connection with the marked rise of both lipase and blood fats at the end of the series with the young animals.

Some practical applications of feeding experiments with albino rats. THOMAS B. OSBORNE and LAFAYETTE B. MENDEL.

The method of feeding which we have used with success in demonstrating the relative nutritive value of the different proteins for either growth or maintenance has been employed for determining the value of several of the concentrated feeding stuffs which are largely used as protein supplements in the rations fed to cattle and other domestic animals. These products have heretofore been valued solely on the basis of the *amount* of protein which they contain, no attention having been paid to the qualitative character of the protein.

[1] Bloor: Jour. Biol. Chemistry, 1914, xvii, 377.

[2] Loevenhart: Am. Jour. Physiology, 1902, v, 334.

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In supplementing a diet of which corn or corn meal forms the chief constituent our experience indicated that better results would be obtained if the protein concentrate contained protein rich in tryptophane and lysine. The results of the experiments thus far conducted have shown this to be true, and plainly indicate that economies can be effected by using proper combinations of these relatively expensive food products. Comparisons already have been made by this method of such products as distillers' grains, brewers' grains, cotton-seed meal, fish meal, and beef meal. It is intended to continue these investigations and extend them to other largely used commercial products.

The influence of chemical substances on immune reactions with special reference to oxidation. AARON ARKIN.

Sodium iodoxybenzoate, an organic peroxide with physiologically active oxygen, has a marked germicidal action toward B. coli, typhosus, pyocyaneus and Staph. aureus, which differs for the different organisms.

Its action is most marked toward the organism containing the least catalase. These results suggest a relationship between catalase value of bacteria and their susceptibility to oxidizing agents.

The compound has a stimulating effect on phagocytosis in vitro. It stimulates the production of antibodies in immunized animals (hemolysin, agglutinin). It has an inhibitory effect on the local tuberculin reaction in tuberculous animals, and also reduces the toxicity of tuberculin in vitro. The substance does not influence the catalase value of the blood. A study of its effect on the catalase value of the tissues is now under way. These results, in the light of the pharmacological action of the substance, suggest that it stimulates the production of antibodies because of some catalytic effect by accelerating oxidations in the tissues which are the site of antibody formation.

The effect of thyro-parathyroidectomy on the blood coagulation time in the dog. SUTHERLAND SIMPSON and A. T. RASMUSSEN.

The object of this investigation was to determine whether removal of the thyroid gland (including parathyroids) in the dog had any effect on the blood coagulation time. The graphic method of Cannon and Mendenhall for measuring the coagulation time was adopted and found to work satisfactorily.

In all twenty-four animals were used. Two sets of observations under normal conditions were made, with an interval of a few days between, to find out whether the time varied appreciably from day to day. Then thyro-parathyroidectomy was performed and when the symptoms of parathyoid tetany appeared the coagulation time was observed again. The results show that no marked effect is produced on the coagulation time by the removal of these glands.

Detection with the string galvanometer of afferent impulses in the brain-stem and their abolition with ether anaesthesia. A. FORBES and R. H. MILLER.

The effect of ether anaesthesia must depend on the blocking of nerve impulses somewhere in the chain of neurones leading from sensory

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receptor to motor end organ. Our experiments have sought to localize the action in some measure, and in particular to determine by means of action currents whether or not ordinary surgical anaesthesia blocks afferent impulses resulting from peripheral stimulation at the synapses through which they pass on their way to the cerebral cortex.

As a preliminary control it was necessary to ascertain whether in the nerve trunk profound etherization abolishes the action current which serves as an index of the nerve impulse. This point was investigated by one of us with McIntosh and Sefton. It was found that even under etherization pushed to the point of abolishing respiration action currents could be led off from a motor nerve under direct stimulation.

The peripheral afferent neurones extend centrally as far as the medulla oblongata. In view of the results with the nerve trunk we should expect no interruption of the action current by ether before this point. The effect of ether must be noted in some neurones central to the medulla.. To obtain a suitable preparation that could be studied with and without anaesthesia decerebration was necessary.

Our experiments were made on decerebrate cats, observations being made at frequent intervals before, during and after anaesthesia by ether inhalation. Decerebration was performed under deep anaesthesia with the Sherrington decerebrator, and the best results were obtained with a transection at the anterior margin of the anterior corpora quadrigemina.

Action currents were recorded with the Cambridge String Galvanometer. For leading off electrodes we first used the porous "boots," later the gelatin type of Lucas, which are preferable. For stimulation single break shocks from a Berne inductorium were delivered through a pair of Sherrington shielded electrodes applied to the sciatic nerve.

When the leads were applied to the brain-stem the excursions of the galvanometer following the stimuli were small, but they were largest when one electrode was placed at the top of the brain-stem and the other at the bottom, and the direction of the major excursion indicates electrical negativity at the bottom. The procedure finally adopted was to place one electrode on the posterior corpus quadrigeminum on one side and the other at the base of the brain-stem 2 or 3 mm. the other side of the median plane. The stimulus was applied to the sciatic nerve on the same side as the upper electrode.

With this arrangement surgical anaesthesia with ether either greatly reduces the size of the excursions, or altogether abolishes them.

We are not prepared to draw generalizations concerning the degree of narcosis requisite for abolition of these nerve impulses, but we have found that abolition generally occurs long before respiration is interfered with. It is evident that if ether anaesthesia suffices to abolish or greatly reduce the magnitude of impulses in these neurones which arise in the medulla, it is more than likely that any impulse persisting in this region will be abolished in the next set of synapses in the chain leading to the cortex. It may then be fairly concluded that surgical anaesthesia protects the cerebral cortex from incoming nerve impulses.

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A smooth-muscle nerve preparation. C. D. SNYDER.

Cinematograph and lantern demonstration of some effects on lesions of the nervous system. F. H. PIKE.

On the secretory discharge of the pituitary body produced by stimulation of the superior cervical ganglion. V. N. SHAMOFF (by invitation).

Concerning the action of various pituitary extracts on the isolated intestinal loop. V. N. SHAMOFF (by invitation).

The influence of certain cereal foods on the gastric secretion. C. C. FOWLER (by invitation), M. E. REHFUSS (by invitation), and P. B. HAWK.

Changes in the composition of the body of fasting lobsters. SERGIUS MORGULIS.

A note on the contractility of the musculature of the auriculo-ventricular valves. JOSEPH ERLANGER.

Kent[1] has recently described muscular tissue extending from the auricles onto the surface of the auriculo-ventricular valves. An examination of the literature as given by Nicolai[2] shows that muscle had been described in this locality by Reid in 1839, by Kürschner in 1840, by Paladino in 1876 and by Albrecht in 1903. In so far as we are aware, however, no one has recorded having observed contractions of these valve leaflets. The following observation seems therefore to warrant a brief note. It was made some years ago (June 3, 1911) while experimenting with the surviving beef's heart.[3] The heart had been perfused for some time. The ventricles had fibrillated; repeated perfusion with 1 per cent potassium chloride solution had failed to revive the rhythm of the heart; the auricles also had stopped beating. When the heart was opened, the posterior leaflet of the mitral valve was seen to be beating rhythmically. The movements were not very extensive. We were then of the opinion that under natural conditions contractions of such a strength would not play any very decided rôle in the normal action of the valves. The leaflet continued to beat after it had been cut entirely free of the heart. Histological examination revealed a few heart muscle fibers in the valve. This was the only instance in the course of a large number of experiments in which a valve leaflet was observed to be contractile. It would not be safe to draw any inferences as to the function of the musculature of the valve leaflets from an isolated observation that the valve musculature may be contractile, though feebly so, and spontaneously rhythmical, and at a time when the auricles themselves are not beating. Some of the pos-

[1] Proc. Roy. Soc., 1915, lxxxviii, p. 537.

[2] Nagel's Handbuch der Physiologie, 1905, i, 846.

[3] Erlanger: Am. Journ. Physiol., 1912, xxx, 395.

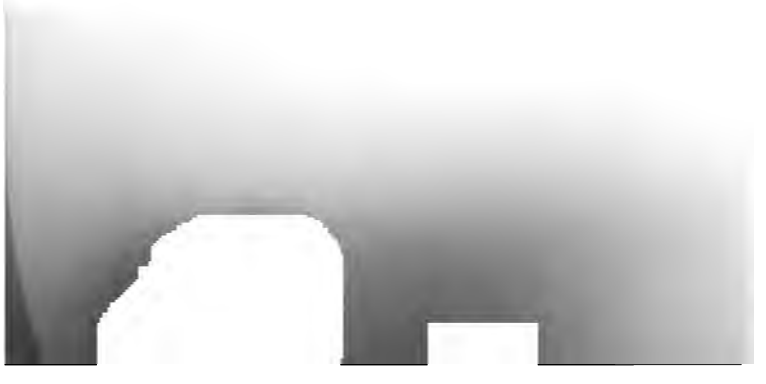
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sibilities in the case are discussed by Nicolai (l. c.) and also by Kent (l. c.). Very recently Dean[4] has noted that before the end of auricular systole, the auriculo-ventricular valves very quickly ascend toward the auricle. Such a movement at this time might well be caused by a contraction of the valve musculature.

The psychic secretion of gastric juice. R. J. MILLER (by invitation), M. E. REHFUSS (by invitation) and P. B. HAWK.

[4] Proc. Soc. for Exp. Biol. and Med., 1915, xiii, 6.

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THE EFFECT OF CASTRATION ON THE WEIGHT OF THE PITUITARY BODY AND OTHER GLANDS OF INTERNAL SECRETION IN THE RABBIT

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INTRODUCTION

The object of the present investigation was to determine the effect produced on the weight of the hypophysis by removing the testes and ovaries from male and female rabbits. It has been reported that the anterior lobe of the pituitary is affected in weight and histological appearance by castration. The same part of the pituitary is generally considered to be in some way associated with body growth. Castration in certain cases has been shown without a doubt to affect growth of animals. With these facts in mind the idea was conceived of utilizing the same animals for a determination of the effect of castration and spaying on the body growth and also the effect on weight or histological appearance of some other glands of internal secretion.

It is probable that the operation which consists of removing the testes from male animals has been practiced longer than any other. Many of the effects have been noticed from the very first and some are still disputed questions. The results most manifest are upon the secondary sex organs and characters; the whole body, however, undergoes changes in metabolism and development. Most of these changes are at best only vaguely understood and of others practically nothing is known. In mammals the most marked change is in the prostate and seminal vesicles. If their development is not complete it ceases, and if complete, atrophy results (Griffiths (9)). The effects which follow

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removal of the ovaries from females are just as distinct, and are probably less understood than those peculiar to males. Some of the results are common to both sexes.

It was not until Brown-Sequard (2) in 1889 published his results that serious investigation began in this field. His findings, although probably not of so much value in themselves, suggested what is to be the basis of explanation for most if not all the effects following the removal of sex organs from both males and females, namely an internal secretion. It seems quite reasonable to suppose that all organs which produce an internal secretion are more or less intimately related to each other and for this reason it is difficult to determine the direct effect upon any one of these by removal or alteration in the normal action of any other.

In certain respects the pituitary gland is considered by some writers to be more closely related to the testes and ovaries than to any other of the glands of internal secretion. Experimentally the relationship between any two of these organs has been studied usually by one or more of four methods, (1) by removing one of the two glands in question and observing the effect produced upon the other in the form of an increase or decrease in weight, histological changes, external appearance and so on; (2) by removing the other gland and noting the reverse effects; (3) by administering to the experimental animal in some form the substance of the gland which in the other cases was taken away; and (4) by reversing this procedure by feeding the substance of the gland upon which the effect was watched in the preceding cases. Another method which can scarcely be placed in any one of these four classes is to remove an organ from its natural position and graft it into some other part of the body. This method has been used to support the theory of internal secretions in the case of the testes of fowls (Foges (8)) and many other forms. In addition to being studied experimentally they have been approached from the field of pathology, from clinical records, and from the results of operations upon the human subject.

Advantage has been taken of most of these methods for adding to our knowledge concerning the relationship between the pituitary and the generative glands. Several investigators have removed the testes or ovaries from various animals under different conditions and have used different methods for determining the effect upon the weight of the hypophysis or other organs. The animals used have included rabbits, guinea pigs, cocks, bulls, buffaloes, sows, ewes, rats, horses and in addition observations on eunuchs. The conclusions thus far reached have

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been contradictory so that at the present time the question is obviously an open one.

With the object of the present experiment in mind, precaution has been taken to avoid all errors which might possibly affect the results. Two series of animals each composed of both sexes have been used.

PREVIOUS WORK

Comparatively few investigators have studied this problem directly. Much however has been written on subjects closely associated with this question and for a complete survey of the literature the reader may consult Biedl (1), D. Noel Paton (20), and Swale Vincent (24). Only those whose results are most closely related to this subject will be mentioned here.

The first and probably the most widely quoted worker in this field is Fichera (7) who observed the weight and histological appearance of the pituitary bodies of 50 cocks and 50 capons, 2 normal and 3 spayed rabbits, 2 normal and 3 spayed guinea pigs, 5 normal and 5 castrated buffaloes and 5 normal and 5 castrated bulls. The figures which he gives include for each group the average, minimal, and maximal weight of the hypophysis in centigrams, for both control and experimental animals. Fichera concludes from these experiments that the pituitary enlarges to about twice the normal size after castration, and also reports that he was able to rapidly reduce this enlargement by the injection of testicular extract. He believes that there is some sort of compensatory relationship between the genitals and the hypophysis. It seems entirely possible however that his figures do not show what might be found if some of his sources of error were eliminated. With the exception of the cocks and capons the number of animals is too small to be taken as conclusive proof when such great individual variations are obtained normally. The body weight of the animals is not given in any case; thus his results are not given in terms of body weight, which obviously might make an appreciable difference. In the case of rabbits and guinea pigs, where females were used, no mention is made as to whether or not the animals had ever been pregnant, and this condition is claimed by some investigators to cause an enlargement of the pituitary gland which probably returns to normal during lactation but may persist for a longer time. The animals are not said to be of the same litter which would also be apt to cause a variation. In case of some animal species, especially those in which the gastro-intestinal con-

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tents may vary between comparatively wide limits in proportion to body weight, it seems necessary to also avoid this possible source of error.

In regard to the condition of the pituitary during pregnancy as just mentioned, Comte (4) seems to have been the first to report an enlargement, and later many others have come to the same conclusion. Erdheim and Stumme (6) have produced indisputable evidence of hypophyseal hypertrophy during pregnancy and of changes which take place in the structure of the organ, similar to those which Fichera, as just mentioned, has described as resulting from castration.

As to the necessity of taking into account the weight of the gastro-intestinal contents in the case of the rabbit, as here considered, reference should be made to some results of Joseph (15) in which the content weight is on the average about 10 per cent of body weight and varies between comparatively wide limits. The same is shown to be true in case of the rabbits used in the present experiment as already published (17).

Cimorini (3) confirms the work of Fichera by using young dogs and states that the changes which take place are similar to those occurring after removal of the thyroids. Tandler and Gross (23) show that there was an anatomical enlargement of the sella turcica by examination of the skulls of skeletons and by means of X-ray photographs of living castrated animals.

The work of Marrassini and Luciani (19) owing to its scope and character undoubtedly deserves careful consideration. Their observations were made on sheep, cattle, dogs, rabbits, guinea pigs and domestic fowls. The age, time after castration, weight, and in most cases the relation of the animals were known, i.e., whether they belonged to the same or different litters. They give not only the gross weight of the animal, the individual weight of each hypophysis and their relative weights, but also the weight of each brain, kidney, heart, suprarenal gland, liver, spleen, pancreas, thyroid, and the normal generative glands. They conclude that in sheep and cattle the weight of the hypophysis, which normally presents wide individual variations seems not to have undergone modifications capable of constituting a particular characteristic of castrated animals. Although no weights with respect to dogs are given in their tables the statement is made that the weight of the hypophysis in proportion to body weight does not show any constant difference which merits special attention. In rabbits and guinea pigs either male or female they do not

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castration any constant modification deserving mention. The same is said of those males in which a bilateral ligature of the ductus deferens was made, and a ligature of the ductus deferens on one side and castration on the other side. They further say that the difference which one sometimes observes in the weight of the hypophysis as well as in all other organs must, in all probability, depend on special individual conditions, not attributable directly to the suppression or the modification of the function of the sexual glands.

D. Noel Paton (20) in discussing this question, considers it to be proven that hypertrophy of the pituitary is caused by castration as well as by thyroidectomy and is inclined to consider this as an indication that there is a reciprocal relationship between these organs. On the gonads the pituitary is generally believed to have an augmentary action; both have an important influence on growth and development, and when the gonads are removed the pituitary is regarded by some as undergoing what is thought to be a compensatory hypertrophy. It is stated that without the pituitary, complete development of the gonads appears impossible.

Cushing (5) reports that in the case of the majority of adult dogs from which the posterior lobe of the pituitary had been removed the females did not come in heat even when observed for nine months, and the males ultimately showed definite testicular atrophy. The puppies of both sexes remain sexually infantile.

Kuhn (16) examined the pituitaries of castrated horses, mares and stallions. Owing to the difficulty of obtaining stallions for examination he was able to report the pituitary weight of only two. His conclusions are therefore mainly based on the comparison of castrated males and normal females. The sex difference in the horse is not regarded as an appreciable factor. The females used in the investigation had never been pregnant. He concludes that the pituitaries of horses do not respond by an increase in size or volume after removal of the testes. He points to the wide individual variation which he noticed among those of the normal groups and attributes the slight difference between the operated and control animals, which is in favor of the normals, to this cause rather than to any effect of castration.

Hatai (10) reports a distinct sex difference when comparing the rate of increase in weight of the hypophysis during growth in male and female albino rats. This sex difference was noticeable in rats which weighed 50 grams, and became more noticeable as the animals increased in weight. The hypophysis in the adult female was more than twice as

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heavy as in the male in proportion to body weight. In a later publication (11) he states that in the case of the Norway rat this sex difference is very slight and he questions whether or not it is present at all in guinea pigs and rabbits as shown by the results of other investigators. No explanation is given for the sex difference in the case of the albino rat but it is made clear by his results that the hypophysis reacts differently according to sex after castration and spaying. In the spayed females the weight of the hypophysis was only slightly greater than that of the controls, amounting to 3.84 per cent which should be considered as within the limits of experimental error and individual variation. The male rats however showed a striking difference in this respect. In the males which had been castrated the hypophysis was 73.62 per cent heavier than in the controls. This enlargement was shown in all of the four groups examined and in each litter. Along with this difference another was noticed, namely that in the females spaying was invariably followed by general overgrowth and obesity, while among the males castration was not followed by these changes. Hatai in a recent contribution (12) further substantiates these conclusions.

Stotsenburg (21) seems to have been the first to make a systematic study of the growth after castration of males among mammals, using the albino rat and concludes by saying "In the case of albino rats, the growth curve for castration is similar to that for normals." As a result of spaying females he reports in a later paper (22) an excess over normals in body length of 3.4 per cent and in body weight of 23.5 per cent and the excess in body length would call for, as he says, an excess in body weight of 12 per cent. Then the difference between 23.5 per cent and 12 per cent must be credited to the deposition of fat. This was confirmed at the post mortem examination. These results as already seen are substantiated by Hatai.

This relationship between the response of the hypophysis on the one hand and general overgrowth on the other has been noticed in the present investigation upon rabbits, the data for which had been recorded before the results of Hatai or Stotsenburg appeared, and will be considered later in this paper.

Some observers as already mentioned explain the hypertrophy of the pituitary following castration as an example of vicarious action. When the sexual glands are removed, the pituitary enlarges to supply some internal secretion to take the place of one which is lacking. Hoskins (14) is of the opinion that the pituitary is normally held in check by the gonads and when this inhibition is removed the pituitary manifests

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increased activity leading to altered metabolism, and thus to an overgrowth of different parts of the body such as occurs both in acromegaly and after castration.

Rosalind Wulzen (25) mentions the evidence of previous workers which is somewhat contradictory regarding the effects on growth following the increase or decrease in amount of anterior lobe secretion which is supplied to different animals either from natural or artificial sources. The preponderance of the evidence cited, however, indicates that the pituitary body either injected or ingested is able to cause a diminution in rate of growth in young animals and that this is due to something more than emaciation has been shown by some who measured the long bones and found a decrease in their length. She also concludes from her own experiments that growth of young fowl is retarded by the addition to the diet of fresh, unmodified anterior lobe of ox pituitary. This was shown in body weight and in length of the long bones, and was more marked in the males than in the females.

MATERIAL

In the experiments here reported the rabbit has been chosen as the experimental animal for the reason that several other observers have used this form and have disagreed as to the results, also because it is inexpensive and at the same time easily obtained and cared for. In all, about 150 rabbits were obtained, of which several were lost in various ways. Since the fact that animals even of the same species raised in different sections of the country may possess marked difference, as is well shown by Marine (18) in case of the thyroids of dogs, it should be stated here that the animals used in this experiment were all born and raised in the neighborhood of Ithaca. Some were bought of farmers, while others were reared in the animal house belonging to the Physiological Department. All animals which seemed to be in any way abnormal were rejected. At the time of operation the animals varied in age from a few weeks to about one year. The body weights ranged from 300 grams to more than 2 kilos as will be seen in the accompanying tables. As to sex they were divided very nearly into two equal groups. Two series of animals have been used, the first including about 60 and the second about 90. The former was observed and examined during 1912 and the latter in the following year. Several objections, which might be raised, became apparent in case of the first series, namely, the animals were not necessarily of the same litter

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although in many cases they probably were. A large proportion was full grown and hence possibly not so suitable for this experiment, and it was not certain that all the females were virgins. For these reasons it was thought advisable to repeat the work on a larger scale, making special effort to control, as far as possible, each operated animal by a normal one of the same litter, and to operate upon all at as early an age as practicable.

METHODS

Relative to the subject of castration and spaying a few general statements may be made. The effect upon the hypophysis following the removal of the testes or ovaries is a difficult question to solve, not because either operation is so complicated, but because of the small size of the hypophysis and its great normal variation as may be seen from the control group. Further difficulty is encountered by the fact that a greater normal variation is apparent when comparison is made between different litters, between animals from different sections of the country, between those which have and those which have not been pregnant, and between animals of different breeds. With these difficulties in mind, precaution has been taken to avoid to the greatest possible degree every source of error, compensating the unavoidable ones by the use of larger numbers of animals than would otherwise be necessary. By an examination of the literature in these particulars a noteworthy fact is revealed that in many experiments which are often quoted the above mentioned precautions were not taken. In some cases the conclusions are drawn from a few operated animals, with an even smaller number as controls, no mention being made as to sex, ratio of hypophysis to body weight, age, time elapsing after operation, or relationship of operated to control animals. These methods lead one to doubt the correctness of the conclusions to which many previous workers have come. Some, however, have worked with great care and this may explain in some respects why their results contradict those of others.

In the present investigation the animals which were purchased from the outside were placed in two pens in order to keep the sexes apart, the individual weight recorded, and a distinguishing number assigned. The color markings which do not change can also be relied upon as a guide to distinction. The litters of rabbits which were born here in our animal house were kept separate until large enough to be marked. They were then weighed as in the above case. Each animal to be operated on was selected whenever possible so that it could be controlled

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by one of the same litter, of approximately the same weight at the time of operation, and always of the same sex. More attention was paid to these points in the second series than in the first.

In the case of young animals which were raised by us, the operation was performed as early as it was regarded advisable with a reasonable degree of safety. Those which were bought from other breeders were usually operated on as soon as received.

In all cases the operation was performed while the animal was under deep ether anesthesia, with aseptic precautions. In the case of the males the testes were removed through a short transverse incision through the skin of the public region. The cord was clamped, cut, and cauterized to obviate the use of a ligature. The ovaries were removed from females sometimes through a median longitudinal incision and sometimes through two opposite lateral incisions in the abdominal wall. After the wounds had been closed a 5 per cent solution of iodine in 70 per cent alcohol was applied occasionally for two or three days and no bandages were used. Only one or two fatalities could possibly be attributed to the operation. Sections of each piece of tissue removed were fixed for future histological examination.

The operated and control animals were kept in the same pen, always in the open air and under precisely the same conditions in every respect. All were well cared for and fed on such food as grass, vegetables, and apples. They were weighed individually on Saturday morning of each week before feeding, and from these weights curves of growth were plotted.

The animals were killed by coal gas. This is not only a convenient and rapid method but was also advantageous in this case because it delays clotting of the blood. The exact weight was now recorded, the bladder emptied by digital pressure and the body weight again taken. The head was now removed and the whole animal allowed to bleed freely, thus reducing the chance of error in weight of the hypophysis and other organs by inclusion of a varying amount of blood.

The next step and the one which required the most careful technique was the removal of the pituitary body. The points to be attended to in this dissection are the complete removal of its surrounding membranes, the severance of the pituitary from the infundibulum at the same point, and these without injury to the organ itself. Different methods of approach were tried on a few of the first series, but it was soon found that the ventral route was preferable. The head being severed the ventral part could be easily removed at the level of the

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mouth by breaking the ramus of the mandible with bone forceps and then extending the angle of the mouth posteriorly by cutting the muscles and skin. The soft palate and mucosa covering the base of the sphenoid bone was then removed, and the position of the pituitary determined by locating the canalis craniopharyngeus which is directly ventral to the pituitary body. The base of the skull was now removed piecemeal by small bone forceps with care not to disturb the tissue in the region of the pituitary body; a small part of the sphenoid bone directly ventral to it being left until all the rest had been removed. When the pituitary is now exposed and the surrounding membranes carefully ruptured the gland will "shell out" when lifted ventrally and anteriorly from behind, from the tentorium sellae in much the same way as the brain can be lifted from the base of the skull from behind when the dura mater is removed from the dorsal surface, and the cranial nerves cut. As the pituitary is lifted out ventrally the infundibulum is flexed and breaks at the same place, in all cases near the pituitary at what is apparently a natural weak point in its structure. The entire body composed as Herring (13) describes of anterior and posterior lobes and pars intermedia, now free, was placed on a small watch glass and transferred immediately to a sensitive chemical balance, where its weight was determined to the tenth of a milligram. The next step in order for each animal was the removal, weighing and fixing of external parathyroids, thyroids, pancreas, spleen, suprarenals, kidneys, thymus, and ovaries if present, or if a male the prostate and also the testes if the animal is a control. Only a small piece of suitable size was placed in the fixing fluid, and the weights determined only for the thyroids, suprarenals, kidneys, testes, ovaries, and uterus. The same procedure was followed in all cases, the primary object being to secure data suitable for purposes of comparison, from both normal and operated animals.

As previously mentioned it was considered necessary to determine for each individual the reduced body-weight. This term will be used throughout to designate the live weight of the animal less the weight of the urine and gastro-intestinal contents, which is found by subtracting the latter from the weight taken immediately after the urine had been expelled. To determine the weight of the gastro-intestinal contents for this purpose, the whole tract from the cardia to the lower end of the rectum was removed with as little of the mesentery as possible and weighed. The contents were forced out by pressure along the outside with a piece of sheet cork which does not cut the tissue, and the

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whole tract again weighed. This weight was then subtracted from the weight of the tract before contents were removed, thus giving the weight of the contents.

From the figures thus obtained the weight of the hypophysis in proportion to body-weight was calculated by dividing the weight of the hypophysis in milligrams by the reduced body-weight in kilos. The same method was followed in determining the relative weight of the other organs to body-weight, except in some instances where the weights are given in grams instead of milligrams.

RESULTS OF PRESENT INVESTIGATION

In presenting the results of this investigation the two series will be considered separately. For the first the averages of the body weights as taken each week are given in Tables I to IV for castrated and control males, and for spayed and control females respectively. Tables V and VI show a comparison of these operated and normal animals without regard to sex.

For the second series corresponding figures are given in Tables VII to XII. Among the animals of this series *ten* castrated males could be controlled by *eight* males of the same litters, and from the females *six* could be controlled by *five* of the same litters. The average weights of these are grouped in Tables XIII to XVI.

Curves I to XVI (figs. 1 to 8) are plotted from these averages and are intended to show relative rates of growth of each group.

The primary object of the experiment was to compare averages and individual weights of the pituitaries of operated and control animals of both sexes. When comparisons are made the weight per kilo of reduced body-weight will be used except where otherwise mentioned. These are to be found in their respective columns of Tables XVII to XXVIII. It will be best to follow at the same time the corresponding curves and notice their relation to pituitary weight. Observe that in general when the pituitary responded to the operation by an increase in size, the body-weight of that group did not respond, and vice versa.

To begin with the first series the male group (Tables XVII and XVIII) of *ten* castrated and *eight* controls give an average for the pituitary of 11.7 mg. and 10.2 mg. respectively which amounts to a difference of 14 per cent. Accompanying this the two growth curves (I and II, fig. 1) are shown to follow the same general course throughout a period of nineteen weeks.

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For the females (Tables XXI and XXII) the spayed group of *twelve* show an average of 12.7 mg. as compared with 13.5 mg. for the *eleven* normals, or a difference of 6 per cent in favor of the latter. The curves (III and IV, fig. 2) show a remarkable difference in that the spayed animals increase in weight much faster than the normals. A part of this difference is evidently due to the fact that the normal animals were larger at the beginning of the experiment, but it scarcely appears reasonable to attribute the whole difference to this cause.

Taking the whole series (Tables XIX and XX) of *twenty-two* operated animals and *nineteen* controls without regard to sex the respective pituitary weights are 12.2 mg. and 12.1 mg., a difference of less than 1 per cent. The curves (V and VI, fig. 3) are resultants of those already mentioned and apparently show a gain in body-weight by the operated, accompanied by a negligible gain by the pituitary.

Consider now the second series and we find the male group (Tables XXIII and XXIV) composed of *twenty-one* castrated and *sixteen* controls with corresponding pituitary weights of 16.1 mg. and 14.6 mg. or a gain of 9 per cent by the castrated animals. With this gain in mind note that there is practically no overgrowth of the castrated animals in body-weight as shown by curves VII and VIII (fig. 4).

For the *nine* spayed females and *eleven* normals (Tables XXVII and XXVIII) the average pituitary weights are 16.1 mg. and 13.2 mg. respectively, making a difference of 23 per cent gain by the operated animals. Accompanying this marked gain the curves (IX and X, fig. 5) show that the spayed group not only did not gain over the normals but actually lost in comparison in body-weight.

When we group the second series without regard to sex (Tables XXV and XXVI) we have *thirty* operated animals with an average pituitary weight of 16.1 mg. and *twenty-seven* controls whose pituitaries average only 14.1 mg. showing a gain of 14 per cent after operation. The curves (XI and XII, fig. 6) by the same grouping show a striking similarity.

The grouping which should probably be considered the most conclusive is the one in which the experimental animals were each controlled by an animal of the same litter. The averages of these are given in Tables XXIII and XXIV for males and Tables XXVII and XXVIII for females and are marked thus *. *Ten* castrated males with an average pituitary weight of 15.2 mg. are in this way compared with *eight* controls with a corresponding weight of 15.6 mg. which is about 3 per cent in favor of the latter. The corresponding curves (XIII and XIV, fig. 7) do not show a marked increase by the castrated animals over the controls but toward the end of the period it may be noticed

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that the castrated animals are about three weeks in advance of the normals which indicates a distinct gain.

In case of the females by this grouping (Tables XXVII and XXVIII) an increase of 25 per cent by the spayed is to be seen from 16.4 mg. and 13.1 mg. for the *six* spayed and *five* control animals respectively. A reverse is noticed in the curves (XV and XVI, fig. 8) from what is shown by the males in the two preceding curves and accompanying this a reverse in the pituitary weights as well.

These results shown in the several different groupings agree with the conclusions of Hatai for the albino rat in that when the body responds by an increase in weight even though slight the pituitary does not show a compensatory hypertrophy, while on the other hand, when the animals which were deprived of their sexual glands do not show an overgrowth there is a distinct, though in no case a very marked, increase in the weight of the pituitary. The rabbits in this case show more of a tendency on the part of the females to a hypophyseal hypertrophy, while in the albino rat according to Hatai the marked hypertrophy was noticed in the males alone.

The question may arise as to whether or not the hypertrophy might not be constant among those animals which do show an increase in body-weight as well as among those which do not, and that by the overgrowth of the body the hypertrophy of the former is not apparent when given in milligrams per kilo of reduced body-weight. An examination of the data however shows that this is not the case, for if the gross weights of the pituitaries be taken instead of the per kilo weights the results will be found to be practically the same.

The evidence set forth by former investigators show that in all probability a hypo-secretion of the pituitary produces an abnormal accumulation of fat, possibly due to lowered oxidation. According to Hatai (10) if a compensatory growth of the hypophysis does not follow, as in the case after spaying of albino rats, the secretion of the unchanged gland must be used for two purposes; first, to replace the ovarian hormone and second for the normal uses whatever these may be. Removal of the sex glands thus seems in some cases to overtax the normal pituitary with a result similar to that of hypo-secretion. In case of hypertrophy of the gland there seems to be no results, attributable to a decreased supply of the secretion, in the form of overgrowth or obesity. Semi-spaying in albino rats seems to produce further evidence in support of this view, since this operation produces neither a change in weight of hypophysis nor an increase in body-weight. The remaining ovary however has been stated to enlarge to twice its normal size and thus prob-

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ably compensates for the lack of secretion from the one which was removed, consequently preventing the changes which would follow if this compensation had to be taken care of by the hypophysis. Be this as it may, it appears for the present to offer a reasonable explanation for the results obtained by comparing the growth curves with the corresponding pituitary averages.

Erdheim and Stumme (6) attribute the enlargement of the hands and lips which are sometimes observed in pregnancy to a hypersecretion of the pituitary. It seems reasonable to regard the hypertrophy during pregnancy as an extra stimulus to growth which is used in the development of the embryo, and that the persistence after parturition may serve as a galactagogue for the further increase in growth of the young after birth.

The difference in the manner in which the pituitary reacts to castration and spaying in different species and even in the same species as reported by different observers may possibly be explained otherwise than by assuming that some have been mistaken in their conclusions. The gland may in some cases be capable of taking on an additional amount of work, or at other times the generative glands might not be producing a secretion at the time of removal which would subsequently have to be produced by some other organ. Another explanation of the variations in results might lie in the possibility of accessory particles of the gland as reported by some as having been separated off along the path of development. These accessory portions may respond by an increase in activity, hence making it unnecessary for the pituitary itself to show any change. In still other species there may be no relation between the hypophysis and the generative glands. Some writers as already mentioned favor the theory that the generative organs have an inhibitory action on the pituitary and when these are removed the pituitary is freed from this inhibition, and consequently increases in size and activity.

In regard to the other organs which were examined the uterus may be mentioned first. In Tables XXVII and XXVIII the weights are shown to be 0.62 gram and 1.32 grams respectively for spayed and control females. One of the group, however no. 64, was apparently in heat at the time she was killed, and this explains the unusually heavy weight of the uterus. Excluding this one the average for the control group is 0.95 gram thus making the uterus of the spayed rabbits average 35 per cent less than for the normals.

As to the heart, no effect can be said to follow castration or spaying. Attention should however be called to the averages given in Tables

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XXIII and XXVIII which show that the smaller animals in general have a larger heart, in proportion to reduced body-weight, than the larger animals. This has also been mentioned in a previous report in case of the gastro-intestinal tract and gastro-intestinal contents (17).

The kidney weights, free from the capsules as given in Tables XXIII to XXVIII, are slightly less for operated than for control animals of both sexes. In case of the males this is in accord with what might be expected because the castrated animals are the heavier, but it does not hold true for the females since the normals are heavier. The difference however is probably too small considering the variation among individuals to justify any claim to an effect following castration or spaying.

Weights for the thyroids are given in Tables XXIII to XXVIII. For females the slight excess shown by the spayed animals is well within the limits of experimental error and individual variation. The whole group of normal males when compared with the whole group of castrated animals shows about 25 per cent in favor of the former, but when a comparison is made in like manner between those selected from the same litters this difference is reduced to less than 10 per cent. It is possible that this apparent reduction in weight of the thyroids is due to castration, but when we notice that among the normal males this organ varies from 52 grams to 91 grams we find ourselves in doubt about a definite conclusion.

The results obtained by previous investigators as to the effect on the suprarenal glands produced by castration and spaying in different animals is briefly summed up by Hatai (12). The results of different observers conflict, but most of them agree that the suprarenals and sexual glands are closely related and that an effect is produced on the former by castration and spaying. The females of the present experiment show no effect except by grouping animals of the same litters. In this manner a gain of 9 per cent is shown by the spayed animals. The whole series of castrated males show a gain of 20 per cent compared with normals, but by selecting those of the same litter for comparison this difference is reduced to only 6 per cent. By comparing the operated animals of the whole series with all the controls, without regard to sex, a gain of 14 per cent is shown by the operated animals. This organ also shows a high degree of variation which however is probably not enough to offset the apparent increase in size following the removal of the sexual glands especially in the case of the male rabbits.

The thymus gland, owing to the large amount of fat inseparably connected with it, was not weighed.

An attempt was made to weigh the pineal gland of each animal, but

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on account of its small size and the difficulty of accurate dissection of fresh specimens only a few weights were taken.

The weights of testes and ovaries for normal animals are recorded in Tables XXIV and XXVIII respectively.

No histological examination of the tissue preserved has yet been made.

CONCLUSIONS

From the results of the experiments described, the following conclusions for the rabbit may be drawn.

1. There is no constant sex difference in the weight of the hypophysis.
2. Neither males nor females show a constant hypophyseal hypertrophy following castration or spaying.
3. The females may be regarded as showing a more constant response by the hypophysis after spaying than is to be seen among the males after castration.
4. From the curves of growth corresponding to each group there is a constant relationship between the rate of increase in body-weight and the response of the hypophysis to castration or spaying.
5. There is less hypertrophy of the hypophysis in those groups which show an increase in rate of growth.
6. In groups where no effect can be shown upon the rate of growth a distinct hypertrophy of the hypophysis is constant though in no case is it very marked.
7. A marked atrophy of the uterus follows removal of the ovaries from females.
8. No change in the weight of the heart or the kidneys can be attributed to castration or spaying.
9. No change can be demonstrated in the thyroid with the possible exception of a moderate decrease in males after castration.
10. The suprarenals show no marked effect. In the males a tendency toward enlargement follows castration, which does not appear after spaying females.
11. No conclusions were reached as to the effect of castration or spaying on the thymus, or pineal gland.

It is with a feeling of true gratefulness that I wish to express in conclusion my thanks to Prof. Sutherland Simpson for providing the material with which this investigation has been carried out, for his many valuable suggestions, and encouragement at all times.

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SERIES NO. I		TABLE I AVERAGE WEIGHT OF CASTRATED MALES	TABLE II AVERAGE WEIGHT OF CONTROL MALES	TABLE III AVERAGE WEIGHT OF SPAYED FEMALES	TABLE IV AVERAGE WEIGHT OF CONTROL FEMALES	TABLE V AVERAGE WEIGHT OF OPERATED MALES AND FEMALES	TABLE VI AVERAGE WEIGHT OF CONTROL MALES AND FEMALES
Number of animals		10	8	12	11	22	19
May	18	1.295	1.312	0.911	1.560	1.127	1.380
	25	1.355	1.359	0.984	1.583	1.199	1.416
June	1	1.378	1.359	0.992	1.536	1.210	1.404
	8	1.411	1.433	1.031	1.559	1.245	1.463
	15	1.454	1.489	1.076	1.587	1.301	1.489
	22	1.498	1.563	1.128	1.620	1.377	1.543
	29	1.563	1.613	1.159	1.647	1.398	1.592
July	6	1.628	1.666	1.199	1.701	1.448	1.639
	13	1.678	1.703	1.246	1.717	1.499	1.670
	20	1.739	1.745	1.295	1.732	1.555	1.689
	27	1.896	1.826	1.471	1.835	1.728	1.779
August	3	1.942	1.863	1.550	1.868	1.794	1.820
	10	1.999	1.918	1.679	1.905	1.878	1.870
	17	1.999	1.941	1.699	1.894	1.865	1.873
	24	2.014	1.984	1.753	1.895	1.930	1.916
	31	2.132	2.083	1.925	2.017	2.069	2.026
September	7	2.199	2.190	2.018	2.060	2.165	2.099
	14	2.241	2.199	2.095	2.105	2.214	2.126
	21	2.318	2.240	2.199	2.169	2.296	2.180
	28	2.378	2.234	2.266		2.362	2.216

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SERIES NO. II	TABLE VII AVERAGE WEIGHT OF CASTRATED MALES	TABLE VIII AVERAGE WEIGHT OF CONTROL MALES	TABLE IX AVERAGE WEIGHT OF SPAYED FEMALES	TABLE X AVERAGE WEIGHT OF CONTROL FEMALES	TABLE XI AVERAGE WEIGHT OF OPERATED MALES AND FEMALES	TABLE XII AVERAGE WEIGHT OF CONTROL MALES AND FEMALES	TABLE XIII AVERAGE WEIGHT OF CASTRATED MALES	TABLE XIV AVERAGE WEIGHT OF CONTROL MALES	TABLE XV AVERAGE WEIGHT OF SPAYED FEMALES	TABLE XVI AVERAGE WEIGHT OF CONTROL FEMALES
Number of animals.....	21	16	9	11	30	27	10	8	6	5
Weight when operated...	1.161	1.181	1.177	1.049	1.166	1.133	0.887	0.869	0.886	0.865
June 28 ..	1.183	1.202	1.123	1.064	1.165	1.146	0.910	0.889	0.833	0.901
July 5. .	1.278	1.316	1.194	1.123	1.253	1.232	1.055	1.031	0.885	0.999
12. .	1.379	1.420	1.302	1.300	1.356	1.371	1.137	1.056	0.993	1.148
19 ...	1.385	1.442	1.332	1.348	1.376	1.404	1.167	1.084	1.086	1.206
26	1.402	1.477	1.409	1.428	1.404	1.458	1.226	1.121	1.133	1.311
August 2 . .	1.484	1.519	1.468	1.520	1.479	1.519	1.319	1.232	1.190	1.390
9. .	1.539	1.588	1.551	1.615	1.542	1.598	1.377	1.320	1.273	1.487
16. . . .	1.635	1.642	1.614	1.691	1.628	1.662	1.482	1.346	1.367	1.561
23.	1.701	1.700	1.670	1.768	1.692	1.727	1.572	1.415	1.492	1.639
30.....	1.764	1.754	1.718	1.864	1.750	1.799	1.649	1.474	1.500	1.725
September 6. . .	1.792	1.766	1.792	1.903	1.792	1.821	1.664	1.508	1.577	1.735
13...	1.835	1.787	1.835	1.986	1.834	1.868	1.702	1.529	1.640	1.831
20.	1.875	1.812	1.883	2.032	1.877	1.901	1.779	1.585	1.688	1.849
27.	1.948	1.875	1.914	2.027	1.937	1.940	1.868	1.676	1.726	1.830
October 4. . .							1.897	1.758		
11.							1.961	1.811		

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TABLE XVII

SERIES NO. 1	NUMBER OF ANIMAL	DAYS SURVIVED AFTER OPERATION	WEIGHT IN KILOS WHEN OPERATED	WEIGHT IN KILOS WHEN KILLED	WEIGHT IN KILOS LESS URINE	WEIGHT IN GRAMS OF GASTRO-INTESTINAL TRACT MINUS CONTENTS	WEIGHT IN GRAMS OF GASTRO-INTESTINAL CONTENTS	REDUCED BODY WEIGHT (R. B. W.) IN KILOS	WEIGHT IN MILLIGRAMS OF PITUITARY	WEIGHT OF PITUITARY IN MGS. PER KILO OF R. B. W.	WEIGHT OF THYROIDS IN MGS. PER KILO OF R. B. W.
	3	155	0.425	2.300	2.285	112.0	324.0	1.943	23.0	11.837	75.14
	17	157		2.345	2.345	131.0	379.0	2.066	35.0	16.940	
	19	158		2.390	2.390	140.0	180.0	2.210	32.0	14.479	56.56
	13	148	2.125	2.530	2.515	118.0	239.0	2.276	27.0	11.862	46.56
Castrated	11	185	0.975	2.675	2.640	186.0	340.0	2.300	40.0	17.391	
Males	1	45	3.375	3.000	3.000	176.0	409.0	2.591	15.0	5.789	50.17
	15	208	1.050	2.885	2.885	154.0	270.0	2.615	27.0	10.325	
	5	199	0.875	3.010	3.010	180.0	315.0	2.695	30.0	11.131	
	9	189	0.860	3.165	3.160	188.0	250.0	2.910	22.0	7.560	70.10
	7	187	0.800	3.650	3.650	195.0	312.0	3.338	33.0	9.886	69.50
Average.....			1.311	2.795	2.788	158.0	293.0	2.495	28.0	11.710	61.31

TABLE XVIII

	4			1.700	1.650	114.0	134.0	1.516	14.0	12.25	71.89
	2			2.490	2.490	140.0	515.0	1.976	22.0	11.19	
	10			2.390	2.390	144.0	276.0	2.114	20.0	9.41	
Control	6			2.350	2.350	120.0	226.0	2.124	20.0	9.41	53.69
Males	12			2.450	2.425	128.0	268.0	2.157	20.0	9.27	38.48
	8			2.745	2.725	140.0	150.0	2.575	27.0	10.48	70.29
	16			3.110	3.090	162.0	330.0	2.760	31.0	11.23	63.76
	14			3.350	3.325	144.0	316.0	3.009	26.0	8.64	62.14
Average.....				2.573	2.555	137.0	277.0	2.279	23.0	10.24	60.21

TABLE XIX

Average for operated males and females ...				2.632	2.625	151.0	284.0	2.344	29.0	12.26	58.13

TABLE XX

Average for control males and females				2.396	2.387	135.0	253.0	2.112	26.0	12.12	62.54

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TABLE XXI

SERIES NO. 1	NUMBER OF ANIMAL	DAYS SURVIVED AFTER OPERATION	WEIGHT IN KILOS WHEN OPERATED	WEIGHT IN KILOS WHEN KILLED	WEIGHT IN KILOS LESS URINE	WEIGHT IN GRAMS OF GASTRO-INTESTINAL TRACT MINUS CONTENTS	WEIGHT IN GRAMS OF GASTRO-INTESTINAL CONTENTS	REDUCED BODY WEIGHT (R. B. W.) IN KILOS	WEIGHT IN MILLIGRAM OF PITUITARY	WEIGHT OF PITUITARY IN MGS. PER KILO OF R. B. W.	WEIGHT OF THYROIDS IN MGS. PER KILO OF R. B. W.
	35	68	0 450	0.935	0 935	71.0	146.0	0.789	7.0	8.87	
	23	85	1 350	1 810	1 810	108.0	262.0	1.548	27.0	17.44	
	33	156	0 480	1 890	1 880	135.0	256.0	1.684	23.0	13.66	89.66
	39	89	2 250	2 350	2 340	140.0	364.0	1.976	32.0	16.18	34.41
	27	179	0.465	2 525	2 525	158.0	302 0	2 223	25.0	11.24	32.84
Spayed	29	181	0 920	2 800	2.778	165.0	325.0	2.453	25.0	10.18	
Females	25	175	1.140	2 695	2.675	150.0	215 0	2.460	28.0	11.38	
	43	87	2 170	2 770	2.770	143.0	299.0	2.471	34.0	13.76	33.91
	37	178	0 765	2 875	2.860	184.0	306.0	2.554	28.0	10.96	67.34
	41	26	2.990	3.130	3.130	160.0	335.0	2.795	59.0	21.11	
	21	189	1 500	3.100	3 090	140.0	293.0	2.797	33.0	11.08	55.06
	31	178	0 970	3 075	3.075	180.0	214.0	2.861	20.0	6.99	74.45
Average.....		133	1.287	2.496	2 489	144.0	276.0	2.218	29.0	12.74	55.38

TABLE XXII

	32			0 890	0 890	60.0	134.0	0.756	5.0	6.65	
	26			1.725	1.725	122.0	198.0	1.527	31.0	20.30	
	30			1.950	1.950	140.0	272.0	1.678	14.0	8.34	78.07
	42			2 250	2 250	110.0	222 0	2.028	29.0	14.30	
Control	28			2 415	2.410	160.0	280.0	2.130	21.0	9.85	74.65
Females	38			2 515	2.490	149.0	323 0	2.167	35.0	16.10	66.91
	22			2 635	2.635	126.0	369 0	2.266	25.0	11.03	62.66
	34			2.560	2.550	160.0	250.0	2.300	30.0	13.04	53.48
	40			2.525	2.525	133.0	221.0	2.304	28.0	12.58	46.44
	24			2.575	2.570	156.0	226.0	2.344	29.0	12.37	69.54
	36			2 910	2.910	150.0	395.0	2.515	60.0	23.85	
Average.....				2.268	2.264	133.0	255.0	2.001	28.0	13.50	64.53

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TABLE XXIII

SERIES NO. II	NO. OF ANIMAL	LITTER	DAYS SURVIVED AFTER OPERATION	WEIGHT IN KILOS WHEN OPERATED	WEIGHT IN KILOS WHEN KILLED	WEIGHT IN KILOS LESS URINE	WEIGHT IN GRAMS OF GASTRO-INTESTINAL TRACT MINUS CONTENTS	WEIGHT IN GRAMS OF GASTRO-INTESTINAL CONTENTS	REDUCED BODY WEIGHT (R. B. W) IN KILOS	WEIGHT IN MILLIGRAMS OF PITUITARY	WEIGHT OF PITUITARY IN MGS. PER KILO OF R. B. W.	WEIGHT OF BOTH THYROIDS IN MGS. PER KILO OF R. B. W.	WEIGHT OF BOTH ADRENALS IN GRAMS PER KILO OF R. B. W.	WEIGHT OF BOTH KIDNEYS IN GRAMS PER KILO OF R. B. W.	WEIGHT OF HEART IN GRAMS PER KILO OF R. B. W.
Castrated Males	1		105	1.700	2.200	2.200	120.0	284.0	1.916	27.0	14.091	58.5	0.210	7.270	2.66
	3		106	1.975	2.450	2.450	112.0	180.0	2.270	26.0	11.894	55.5	0.180	6.787	2.48
	5		107	2.200	2.615	2.605	117.0	301.0	2.304	29.1	12.630	49.5	0.159	6.303	2.42
	7		100	1.925	2.050	2.050	114.0	174.0	1.876	30.0	15.943	60.8	0.406	6.215	2.84
	9		108	1.600	2.170	2.160	108.0	244.0	1.916	29.0	15.135	50.6	0.359	5.930	2.95
	11		103	1.310	1.750	1.740	96.0	168.0	1.572	24.6	15.666	52.8	0.237	5.203	2.74
	13		103	1.650	1.475	2.470	132.0	278.0	2.192	29.0	13.270	75.3	0.178	7.303	2.83
	15		101	1.725	2.225	2.225	117.0	232.0	1.993	29.0	14.601	43.7	0.296	6.126	2.56
	17	A*	112	1.275	2.400	2.400	152.0	272.0	2.128	30.6	14.379	52.2	0.337	5.430	3.05
	19	A*	112	1.275	2.500	2.475	144.0	280.0	2.195	32.0	14.578	60.1	0.222	6.300	3.05
	21	B*	112	1.025	2.170	2.160	106.0	322.0	1.838	23.0	12.513	62.0	0.114	8.890	3.18
	23	B*	112	0.900	1.825	1.800	118.0	176.0	1.642	24.8	15.364	70.2	0.168	7.881	3.17
	27	B*	112	0.850	1.750	1.740	102.0	270.0	1.470	23.0	15.646	54.4	0.170	8.823	3.22
	29	A*	127	0.900	2.540	2.525	141.0	383.0	2.142	35.4	16.526	57.4	0.307	8.235	3.03
	33	C	127	0.480	1.800	1.800	106.0	239.0	1.561	33.0	21.140	83.3	0.295	9.122	3.47
	35	C	127	0.430	2.200	2.200	120.0	380.0	1.820	44.6	24.505	61.5	0.238	8.247	3.23
	37	D	127	0.525	1.320	1.300	84.0	158.0	1.142	31.4	27.496	79.7	0.688	10.998	3.71
	41	E*	102	0.890	1.615	1.615	90.0	256.0	1.359	28.0	20.603	88.3			
	45	E*	123	0.855	2.050	2.050	115.0	185.0	1.865	26.0	13.941	62.2	0.165	7.501	3.03
	46	E*	125	0.625	2.220	2.210	122.0	281.0	1.929	24.6	12.752	50.8	0.140	6.526	2.96
	47	F*	125	0.375	1.725	1.710	117.0	273.0	1.437	23.0	16.005	66.1	0.177	7.702	3.05
Average				1.161	2.049	2.090	116.0	254.0	1.836	28.7	16.127	61.6	0.252	7.339	2.98
Average*			116	0.887	2.182	2.169	120.7	269.8	1.801	27.0	15.231	62.4	0.200	7.476	3.08

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TABLE XXIV

No. of Animal	Litter	Date of Weaned after Operation	Weight in Kilos before Operation	Weight in Kilos when Killed	Weight in Kilos	Weight in Grams of Gastro-intestinal Tract Minus Contents	Weight in Grams of Gastro-intestinal Contents	Reduced Body Weight (R. B. W.) in Kilos	Weight of Pituitary in Milligrams	Weight of Pituitary in Mgs. per Kilo of R. B. W.	Weight of Both Thyroids in Mgs. per Kilo of R. B. W.	Weight of Both Adrenals in Grams per Kilo of R. B. W.	Weight of Both Testes in Grams per Kilo of R. B. W.	Weight of Both Kidneys in Grams per Kilo of R. B. W.	Weight of Heart in Grams per Kilo of R. B. W.
Control Males															
2	A*		1.700	2.050	2.042	128.0	207.0	1.835	26.5	14.441	61.5	0.193	1.65	7.37	2.89
4			1.825	2.135	2.135	115.0	308.0	1.827	23.8	13.015	74.4	0.235	1.22	8.99	2.91
6			2.175	2.400	2.380	102.0	171.0	2.209	23.6	10.683	64.2	0.161	2.68	7.57	2.62
10			1.550	1.800	1.820	95.0	133.0	1.667	22.2	13.317	57.5	0.252	2.13	8.02	3.51
12			1.575	1.790	1.700	82.0	121.0	1.579	26.8	16.972	77.2	0.320	1.86	6.72	2.72
16			1.850	2.075	2.075	112.0	270.0	1.805	24.0	13.296	59.2	0.282	1.22	6.37	2.74
18	A*		0.900	1.830	1.830	104.0	198.0	1.632	20.0	12.254	64.9	0.333	3.05	9.55	3.62
20	A*		1.350	2.310	2.453	123.0	301.0	2.151	28.6	13.296	52.4	0.173	2.41	7.00	3.35
22	B*		1.050	2.230	2.230	112.0	262.0	1.968	30.8	15.650	58.9	0.129		8.42	3.23
24	B*		0.740	1.530	1.530	100.0	188.0	1.337	19.6	14.659	65.0	0.187		7.91	3.59
26	B*		0.675	1.820	1.840	120.0	288.0	1.502	29.6	19.707	53.9	0.167	2.24	7.88	4.04
30			0.900	2.425	2.375	137.0	263.0	2.112	29.0	13.731	79.0	0.204	2.41	7.39	3.11
42	E*		0.805	1.930	1.930	120.0	278.0	1.652	25.0	15.133	72.0	0.094	2.81	8.56	3.16
44	E*		0.920	2.000	2.050	108.0	267.0	1.783	28.8	16.208	87.4	0.196	1.05	9.63	2.97
48	F*		0.515	1.625	1.580	103.0	262.0	1.328	24.8	18.674	91.8	0.221	1.87	8.75	2.91
51				2.150	2.140	120.0	328.0	1.812	25.4	14.017	65.7	0.209	3.52	10.63	3.00
Average			1.235	2.028	2.004	111.0	242.0	1.762	25.5	14.690	76.8	0.210	2.15	8.17	3.15
Average*			0.870	1.944	1.926	111.0	257.0	1.669	25.9	15.698	68.3	0.188	1.68	8.61	3.36

TABLE XXV

Average for operated males and females			2.052	2.074	2.074	117.0	245.0	2.409	28.5	16.137	61.5	0.242		7.23	2.94

TABLE XXVI

Average for control males and females			2.114	2.103	2.103	114.0	248.0	1.856	25.7	14.117	64.3	0.212		7.07	3.05

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TABLE XXVII

SERIES NO. II	NUMBER OF ANIMAL	LITTER	DAYS SURVIVED AFTER OPERATION	WEIGHT IN KILOS WHEN OPERATED	WEIGHT IN KILOS WHEN KILLED	WEIGHT IN KILOS LESS URINE	WEIGHT IN GRAMS OF GASTRO-INTESTINAL TRACT MINUS CONTENTS	WEIGHT IN GRAMS OF GASTRO-INTESTINAL CONTENTS	REDUCED BODY WEIGHT (K. B. W.) IN KILOS	WEIGHT OF PITUITARY IN MILLIGRAMS	WEIGHT OF PITUITARY IN MGS. PER KILO OF K. B. W.	WEIGHT OF BOTH THYROIDS IN MGS. PER KILO OF K. B. W.	WEIGHT OF BOTH ADRENALS IN GRAMS PER KILO OF K. B. W.	WEIGHT OF BOTH OVARIES IN GRAMS PER KILO OF K. B. W.	WEIGHT OF UTERUS IN GRAMS PER KILO OF K. B. W.	WEIGHT OF BOTH KIDNEYS IN GRAMS PER KILO OF K. B. W.	WEIGHT OF HEART IN GRAMS PER KILO OF K. B. W.
	83	H*	116	0.855	2.075	2.075	130.0	173.0	1.902	22.0	11.56	62.0	0.201		1.23	5.91	2.80
	85	H*	116	0.920	2.120	2.090	124.0	214.0	1.876	23.2	12.36	53.8	0.202		1.58	6.25	2.69
	61		130	1.420	1.955	1.950	98.0	172.0	1.778	26.0	14.62	38.8	0.217		0.63	6.96	3.00
Spayed	65		123	2.125	2.285	2.280	129.0	262.0	2.018	36.2	17.94	53.0	0.279		0.93	6.25	2.88
Females	67		125	1.730	3.020	3.020	152.0	220.0	2.800	39.2	14.00	69.7	0.165		0.41	5.99	2.36
	69	A*	99	0.860	1.330	1.325	123.0	280.0	1.045	26.0	24.88	90.9	0.330		0.14	7.30	3.66
	71	B*	122	1.100	2.500	2.475	126.0	256.0	2.219	31.0	13.97	47.3	0.129		0.39	7.12	2.70
	73	B*	121	0.750	1.970	1.965	118.0	292.0	1.673	26.2	15.66	58.6	0.179		0.17	9.22	3.10
	75	G*	96	0.830	1.275	1.260	75.0	135.0	1.125	23.0	20.44	77.3	0.266		0.15	7.92	3.25
Average					2.059	2.049	119.0	223.0	1.826	28.1	16.16	61.3	0.218		0.62	6.99	2.94
Average*				0.886	1.878	1.865	116.0	225.0	1.643	25.2	16.48	64.9	0.218		0.61	7.29	3.04

TABLE XXVIII

	84	H*			2.050	2.050	130.0	212.0	1.838	18.0	9.79	64.4	0.219	0.038	0.79	6.16	2.46
	79	C			2.300	2.300	126.0	304.0	1.996	29.0	14.53		0.264	0.071		10.06	3.17
	80	C			2.225	2.225	120.0	294.0	1.931	26.2	13.57	69.9	0.328	0.078	0.91	8.41	3.28
	81	C			2.475	2.475	126.0	364.0	2.111	34.0	16.11		0.250	0.079	1.28	8.40	2.92
Control	62				1.930	1.930	88.0	155.0	1.775	25.8	14.54	62.5	0.280	0.101	1.48	6.56	2.81
Females	64				2.590	2.590	110.0	192.0	2.398	31.0	12.93	55.8	0.147	0.218	4.70	6.40	3.12
	66				3.220	3.220	123.0	187.0	3.033	26.0	8.57	46.4	0.130	0.113	1.30	5.66	1.94
	70	A*			2.190	2.180	138.0	382.0	1.798	31.0	17.24	77.8	0.238	0.061	0.77	8.64	3.18
	72	B*			2.455	2.450	132.0	296.0	2.154	24.0	11.14	55.2	0.111	0.058	1.06	8.29	2.87
	74	B*			1.900	1.890	104.0	248.0	1.642	24.0	14.62	37.1	0.174		0.29	6.91	3.01
	76	G*			1.405	1.405	90.0	180.0	1.225	16.0	13.06	54.6	0.257	0.101	0.66	7.63	2.95
Average					2.249	2.246	117.0	255.0	1.991	25.9	13.28	58.2	0.218	0.092	1.32	7.56	2.88
Average*					2.000	1.995	119.0	264.0	1.751	23.0	13.17	57.8	0.200	0.065	0.71	7.53	2.89

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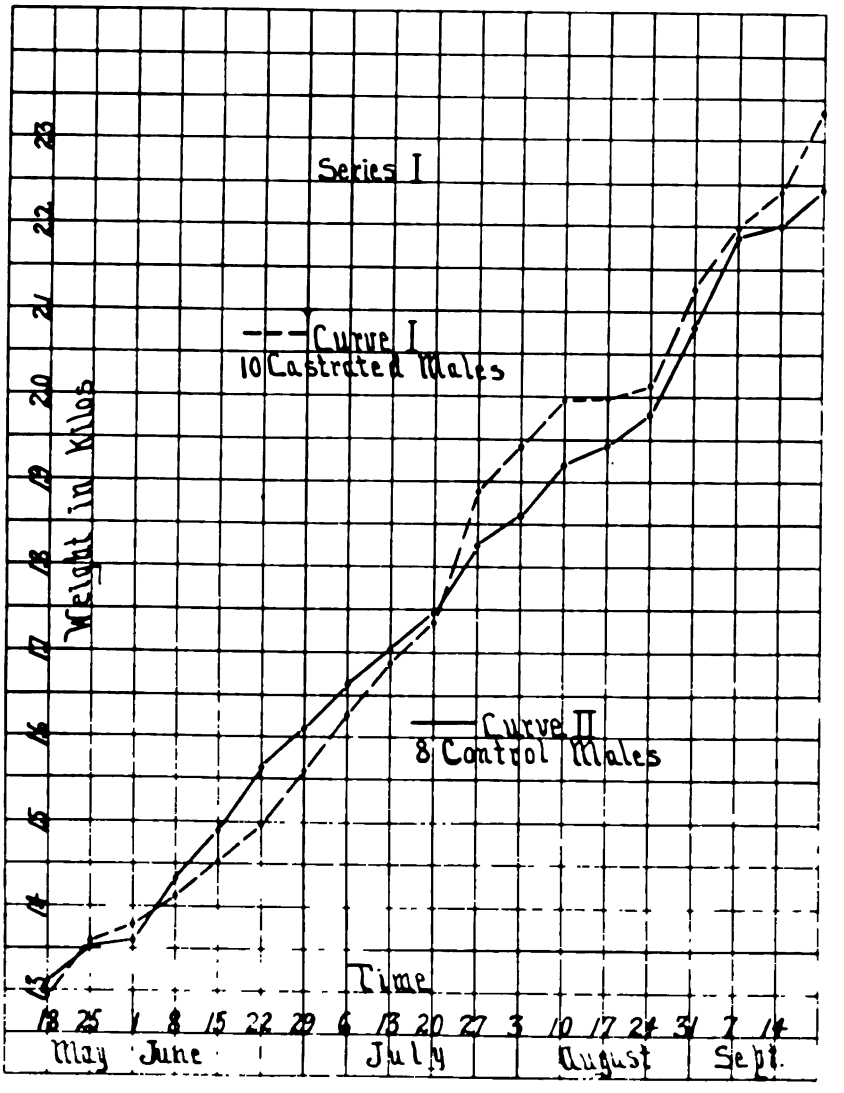


Fig. 1. Curve I (broken line), growth of ten castrated males. Series I. Average pituitary weight per kilo of R. B. W. 11.7 mg.

Curve II (solid line), growth of eight control males. Series I. Average pituitary weight per kilo of R. B. W. 10.2 mg. Difference, 14 per cent.

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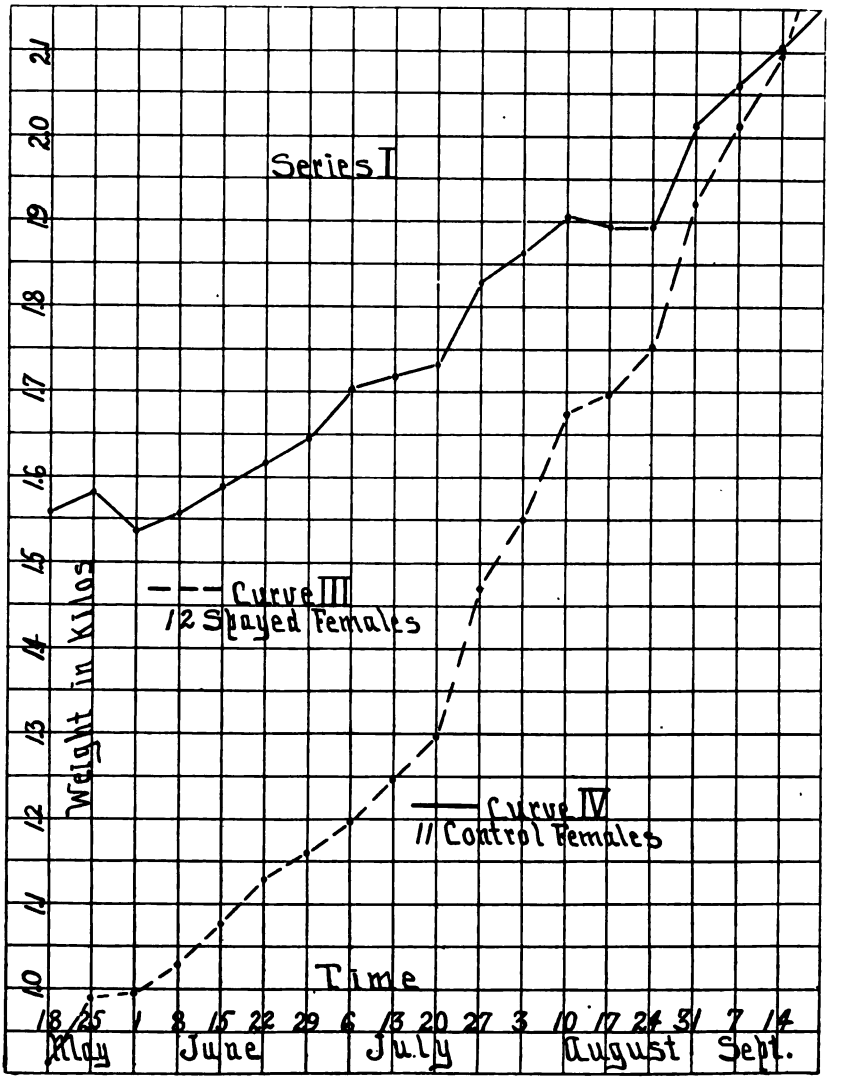


Fig. 2. Curve III (broken line), growth of twelve spayed females. Series I. Average pituitary weight per kilo of R. B. W. 12.7 mg.

Curve IV (solid line), growth of eleven control females. Series I. Average pituitary weight per kilo of R. B. W. 13.5 mg. Difference, 6 per cent.

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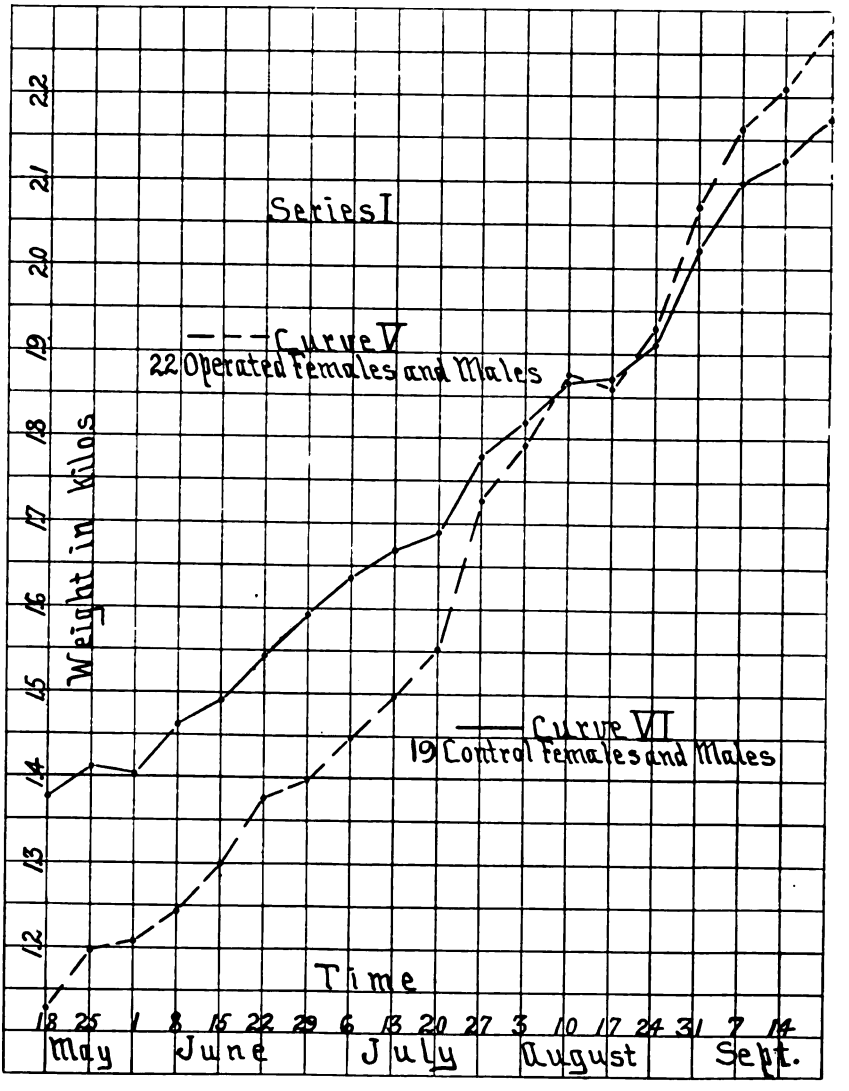


Fig. 3. Curve V (broken line), growth of twenty-two operated animals of both sexes. Series I. Average pituitary weight per kilo of R. B. W. 12.2 mg.

Curve VI (solid line), growth of nineteen controls of both sexes. Series I. Average pituitary weight per kilo of R. B. W. 12.2 mg.

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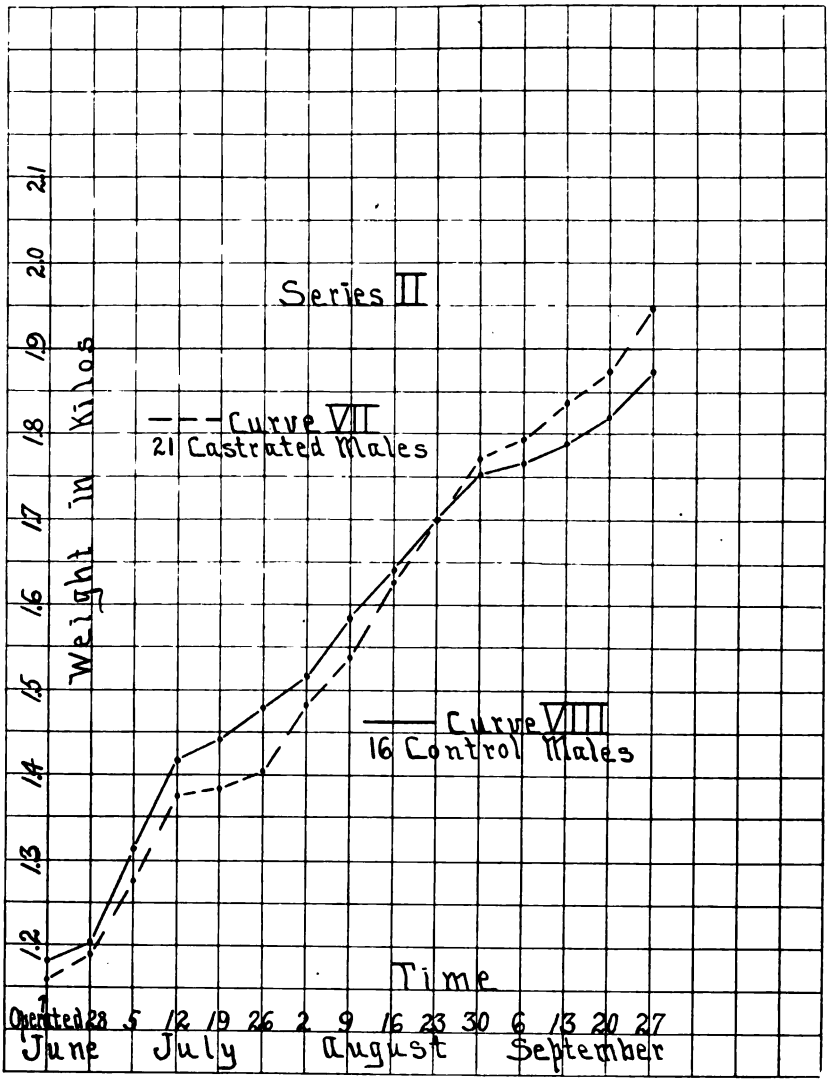


Fig. 4. Curve VII (broken line), growth of twenty-one castrated males. Series II. Average pituitary weight per kilo of R. B. W. 16.1 mg.

Curve VIII (solid line), growth of sixteen control males. Series II. Average pituitary weight per kilo of R. B. W. 14.6 mg. Difference 9 per cent.

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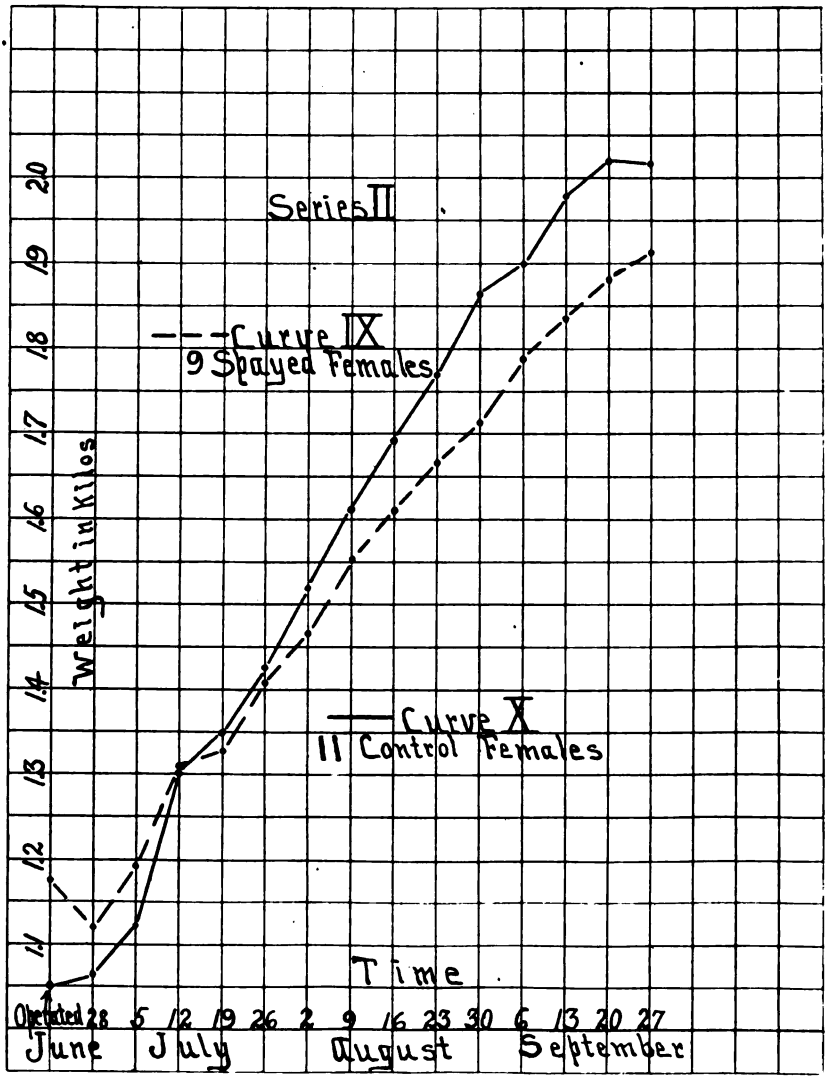


Fig. 5. Curve IX (broken line), growth of nine spayed females. Series II. Average pituitary weight per kilo of R. B. W. 16.1 mg.

Curve X (solid line), growth of eleven control females. Series II. Average pituitary weight per kilo of R. B. W. 13.2 mg. Difference, 23 per cent.

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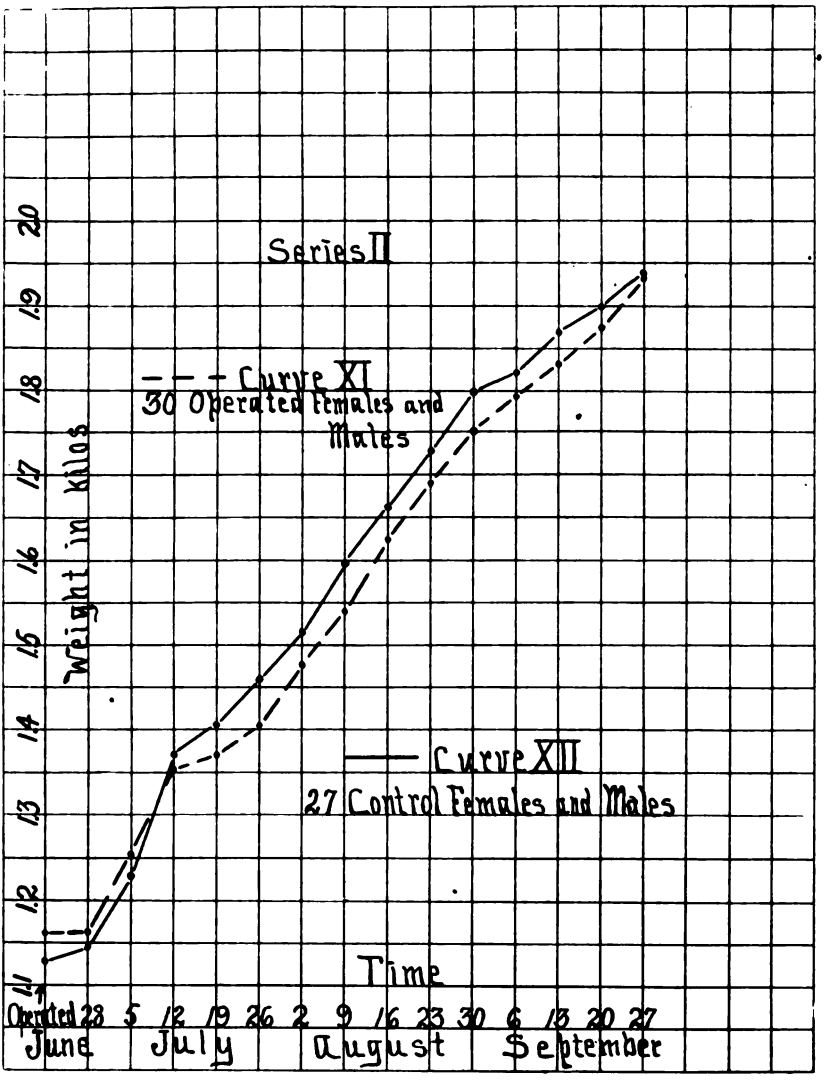


Fig. 6. Curve XI (broken line), growth of thirty animals of both sexes. Series II. Average pituitary weight per kilo of R. B. W. 16.1 mg.

Curve XII (solid line), growth of twenty-seven controls of both sexes. Series II. Average pituitary weight per kilo of R. B. W. 14.1 mg. Difference, 14 per cent.

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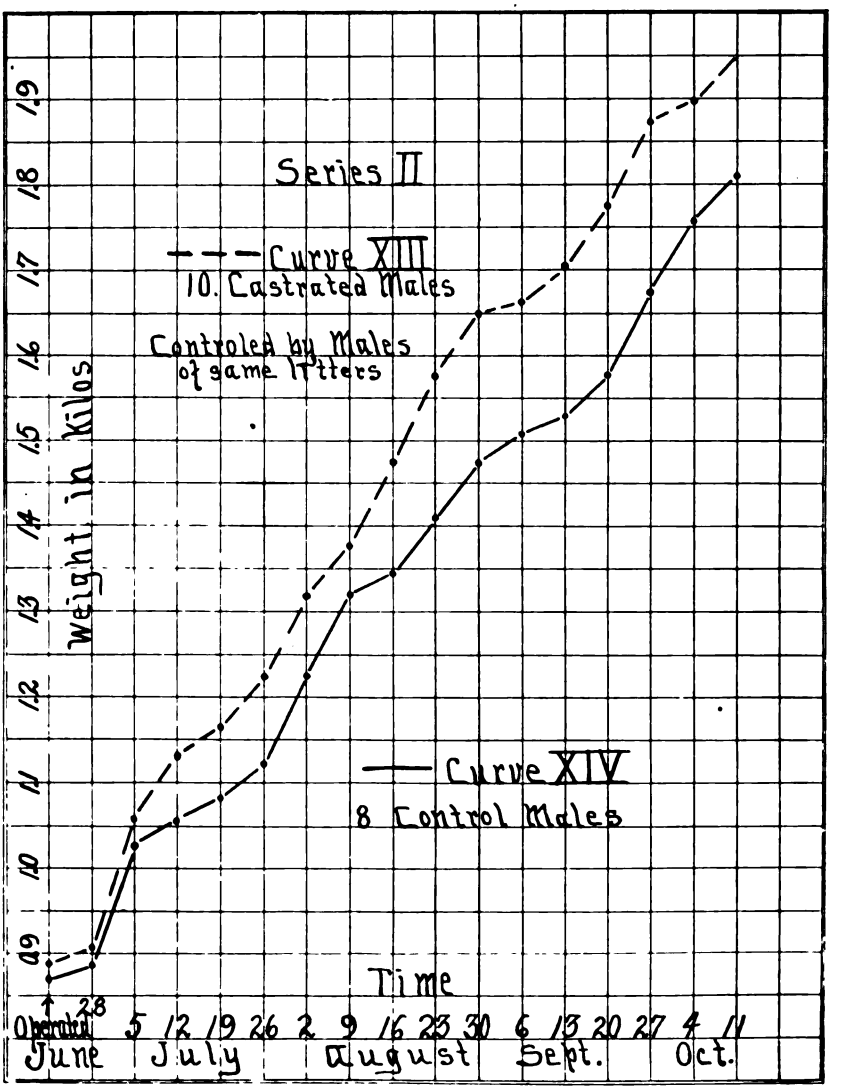


Fig. 7. Curve XIII (broken line), growth of ten castrated males controlled by animals of the same litters. Series II. Average pituitary weight per kilo of R. B. W. 15.2 mg.

Curve XIV (solid line), growth of the eight control males. Series II. Average pituitary weight per kilo of R. B. W. 15.6 mg. **Difference, 3 per cent.**

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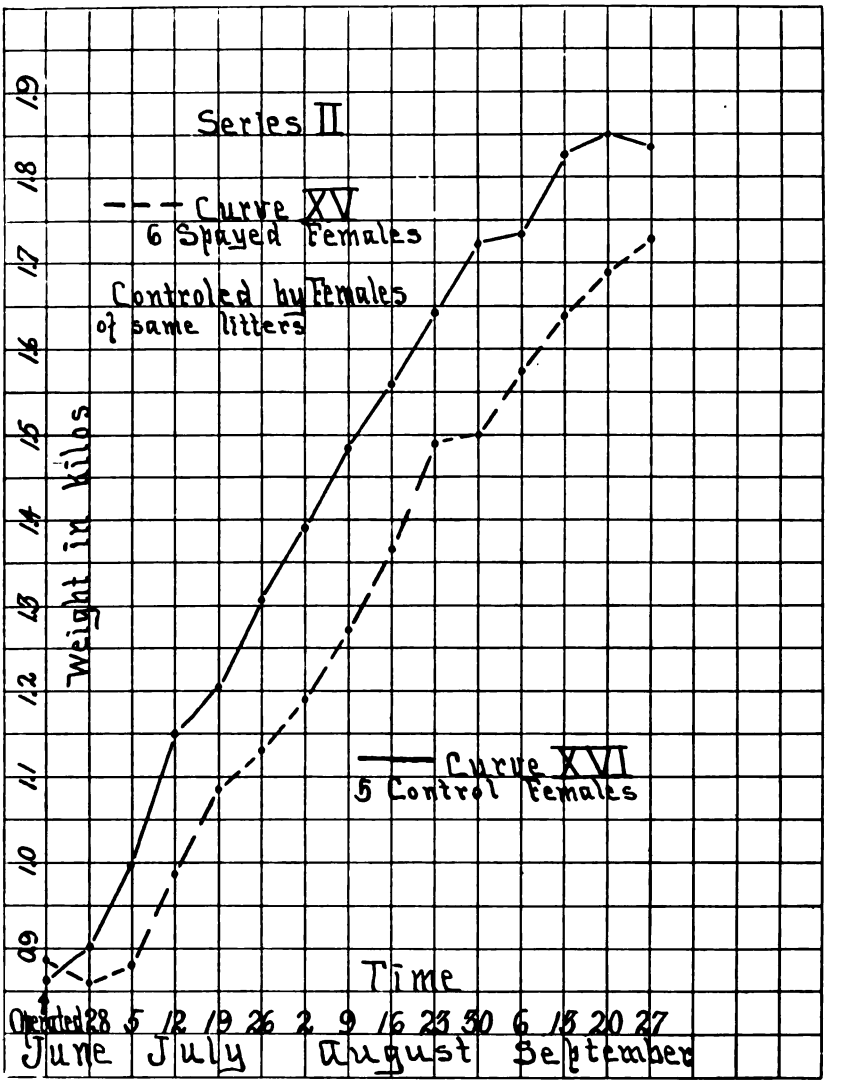


Fig. 8. Curve XV (broken line), growth of six spayed females controlled by animals of the same litters. Series II. Average pituitary weight per kilo of R. B. W. 16.4 mg.

Curve XVI (solid line), growth of the five control females. Series II. Average pituitary weight per kilo of R. B. W. 13.1 mg. Difference, 25 per cent.

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FACTORS AFFECTING THE COAGULATION TIME OF BLOOD

VIII. The Influence of Certain Metals and the Electric Current

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From the Laboratory of Physiology in the Harvard Medical School

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The earliest work on the effect of galvanism on the blood was done as far back as 1824 by Scudamore (1) who distinguished clearly between the effects at the two poles.

> Healthy blood was portioned into two cupping glasses. . . . Thirty pair of plates, four inches square, were employed for the galvanic action. Immediately on introducing the wires, at the negative one a mottled scum appeared, having in color, shades of green, red, and yellow, with a copious disengagement of gas. To the positive wire was attached a dense black coagulum which, as it dried, assumed the appearance of charcoal. . . . In three minutes the galvanized blood afforded a pellicle, when the other portion had not begun to coagulate. A similar difference continued; and at the expiration of nine minutes, the galvanized blood was very much advanced in coagulation, and the other gave only a dense coagulum from the bottom.

Eight years later Benjamin Phillips (2) published his findings on the earliest application of electricity to blood in circulation. In 1867 Duncan and Fraser (3) reported on work done up to that time, and further gave original cases and experiments on electrolysis in aneurism. Somewhat previously Steinlein (4), working with egg white found no change when platinum electrodes and a weak current were used, but found a coagulation at the positive pole when electrodes of oxidizable metals—tin, iron, copper, zinc—were used. Recently Hunner (5) has summarized the cases of aneurism treated by wire and galvanism up to 1900.

During some preliminary experiments we became aware of the remarkable effect of trivalent atoms on the precipitation of egg white and certain blood corpuscles. Mines (6) found that egg white is at once precipitated by a simple trivalent ion such as lanthanum (p. 211), and that the blood corpuscles of Scyllium canicula are at once agglutinated by

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cerium chloride in a solution of 0.0008 molar, though some agglutination is present with a dilution of one-tenth of this (pp. 226, 227). Much higher concentrations of bivalent magnesium and univalent sodium are necessary to produce any agglutination. Consequently we determined to make use of wire of aluminum, a trivalent metal, and to compare it with copper, previously used in coagulation experiments by Cannon, and with iron originally used in wiring aneurisms.

The object of this study then was to determine quantitatively the effect of the three metals on the coagulation time of blood, and to determine the effect of the electric current as it passed into and through blood from the wire as a positive pole.

The method. The method was that devised by Cannon and Mendenhall (7) modified to suit the requirements of the experiment. The recording device consisted of a lever whose long arm wrote on a slowly moving kymograph directly above an electromagnetic time signal. On the short arm of the lever hung the wire which dipped into the blood. The lever was so balanced that the short arm plus the copper wire exceeded the long arm by about 30 mgm. Special weights were used when the aluminum and iron wires (of the same gauge) were employed so that the short arm still exceeded the long arm by 30 mgm. Another lever of sufficient weight rested across the long arm to prevent its rising except at the proper moment, and this second lever was lifted by a simple pulley device, and checked by an equally simple device. This modification was suggested in part by Mendenhall.

Preliminary experiments showed the evolution of gas at the negative pole as spoken of by Scudamore. To separate the poles so that the phenomena would be distinct, an addition was made to the tube and cannula employed by Cannon and Mendenhall. This addition consisted of a short rubber connection which joined the small end of the original cannula to one end of a short U made of the same glass as the cannulae, then another longer rubber connection which joined the other end of the U to a second cannula. The blood was drawn through the second cannula and was permitted to flow through the tubes until the proper amount was present in the original cannula. If a current was to be used, the second cannula was removed to allow easy escape of the gas evolved at the negative electrode. This negative electrode was a simple steel needle soldered to copper wire, and was passed through the second rubber connection just above the glass of the U-tube.

Electricity was drawn from a direct-current lighting circuit, and the amount flowing was easily controlled by a rheostat. From the positive post of the rheostat, the current flowed through a voltmeter, cali-

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brated to read also under the conditions of the experiment in milliamperes. Thence the current passed through a pole changer, to the fulcrum of the recording lever, through the lever itself to the wire at its short end. This contact—lever to wire—was necessarily a loose one, and it was improved at first by amalgamation. This, while satisfactory for the copper, was not for the aluminum, but later the contact was satisfactorily established by the presence of a drop of Ringer's solution. The current then flowed through the wire into the blood in the original cannula, through blood in the constricted neck of the cannula (where considerable resistance was offered), through the blood in the tube to the negative electrode previously described. From here the course was through the pole changer to the negative post on the rheostat. In all the results used in this paper, if current was flowing at all, it was constant—kept so by manipulation of the rheostat if necessary.

Belgian hares, all of the same stock, were used because the coagulation time of their blood was known to be longer than that of cats. They were anæsthetized by urethane, 2.5 grams per kilo of body weight. Blood was drawn from the right femoral artery, just below the deep femoral branch. Other details, as of temperature, were carried out according to the directions of Cannon and Mendenhall.

For the first half minute, required to draw the blood and prepare the apparatus, the conditions were nearly constant. Consequently for the more accurate comparison of the effect of the various factors this half minute was subtracted in each case from the actual coagulation time. This allowed the first record in each case to be taken to prove that conditions were satisfactory. If current was used it was turned on as soon as possible after this test record—practically coincidentally.

Experimental results. The figures given are only those where the technique was unquestioned, and come from the nineteen last experiments of the series. Practically as many more earlier preliminary experiments were performed.

The first figures are arranged to show a comparison between copper, aluminum and iron, when they were used in the ordinary way without the passage of current.

METAL	NUMBER OF OBSERVATIONS	AVERAGE COAGULATION TIME	HIGHEST COAGULATION TIME	LOWEST COAGULATION TIME
		minutes	minutes	minutes
Copper	44	9.6	16 5	5.0
Aluminum	32	5 3	9.5	2.0
Iron	14	8.3	13 5	5 5

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In only two observations did the coagulation take longer with aluminum than with copper in the same blood.

In only two experiments of the nineteen was the average coagulation time with aluminum as great as with copper. In all other experiments the average clotting time with copper was definitely and consistently greater. Iron seemed to act rather like copper than like aluminum.

The average coagulation time with aluminum (5.3 minutes) was reduced from the average with copper (9.6 minutes), when no current was passing, by 45 per cent.

The use of electricity. The rheostat supplied between 35 and 40 volts. Owing to the high resistance (largely due to the constriction

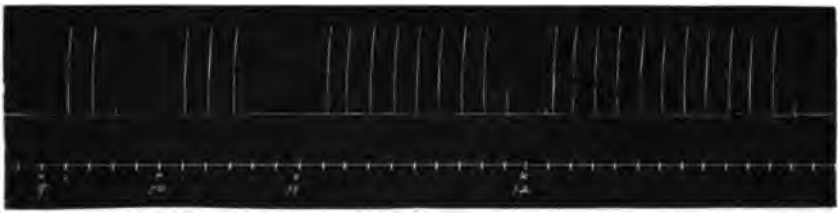


Fig. 1. Experiment 32. Factors affecting coagulation time of blood. The influence of certain metals and the electric current. A series of coagulation times under different conditions. *x*, Blood drawn. . Current on. Time in half minutes. *9*, Aluminum wire, 1 milliampere, time 1.5 minutes. *10*, Copper wire 1 milliampere, time 2.0 minutes. *11*, Aluminum wire, no current, time 4.5 minutes. *12*, Copper wire, no current, time 6 minutes.

at the neck of the original cannula), the current flowing was 1 milliampere under a pressure of 10 volts. At other times the rheostat supplied 70 to 80 volts, and the current flowing was 2 milliamperes under 20 volts pressure. The figures immediately following show the effect of the passage of 1 milliampere of current when copper wire was used as the positive pole.

	NUMBER OF OBSERVATIONS	CURRENT	AVERAGE COAGULATION TIME	HIGHEST COAGULATION TIME	LOWEST COAGULATION TIME
			minutes	*minutes*	*minutes*
Copper.....	44	No current	9.6	16.5	5.0
Copper.....	28	1 milliampere	2.9	5.5	1.0

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In no experiment was the coagulation time, with the current passing, as long as the coagulation time without current.

The average coagulation time with a current of 1 milliampere passing through copper wire was reduced from the average time of copper without current by 70 per cent (see fig. 1).

When aluminum wire was used in place of copper wire, the coagulation times were shorter, but the percentage reduction was about the same.

	NUMBER OF OBSERVATIONS	CURRENT	AVERAGE COAGULATION TIME	HIGHEST COAGULATION TIME	LOWEST COAGULATION TIME
			minutes	*minutes*	*minutes*
Aluminum	32	None	5.3	9.5	2.0
Aluminum	30	1 milliampere	1.4	3.0	0.5

The average coagulation time with a current of 1 milliampere passing through aluminum was reduced from the average time of aluminum without current by 74 per cent.

When 2 milliamperes of current passed instead of one, the coagulation time was reduced still more. With copper the figures were as follows:

	NUMBER OF OBSERVATIONS	CURRENT	AVERAGE COAGULATION TIME	HIGHEST COAGULATION TIME	LOWEST COAGULATION TIME
			minutes	*minutes*	*minutes*
Copper	44	None	9.6	16.5	5.0
Copper	10	2 milliamperes	1.9	3.0	0.5

The average clotting time with a current of 2 milliamperes passing through copper was reduced from the average time of copper without current by 80 per cent.

If aluminum replaced the copper, and the same amount of current was used—2 milliamperes—the coagulation time was shorter, and the percentage reduction almost equalled that with copper.

	NUMBER OF OBSERVATIONS	CURRENT	AVERAGE COAGULATION TIME	HIGHEST COAGULATION TIME	LOWEST COAGULATION TIME
			minutes	*minutes*	*minutes*
Aluminum	32	None	5.3	9.5	2.0
Aluminum	12	2 milliamperes	1.0	1.5	0.5

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The average coagulation time with 2 milliamperes passing through aluminum was reduced from the average time of aluminum without current by 81 per cent. With the present arrangement of the apparatus, owing to the resistance imposed largely by the constriction at the neck of the original cannula, it was impossible to use greater amounts of current than 2 milliamperes. The use of 1 milliampere reduced the average clotting time with copper 70 per cent, with aluminum 74 per cent; the use of 2 milliamperes reduced the average clotting time with copper 80 per cent, with aluminum 81 per cent.

It is of interest to study the effect of the use of two factors causing reduction in clotting time—aluminum and current, as compared with copper without the passage of current.

	NUMBER OF OBSERVATIONS	CURRENT	AVERAGE COAGULATION TIME
			minutes
Copper	44	None	9.6
Aluminum	12	2 milliamperes	1.0

The percentage reduction of the average clotting time under these circumstances—the use of aluminum and 2 milliamperes—from the average time of copper without current was 90 per cent.

The effect of the current, 1 milliampere, passing through the soft iron wire was unexpected in view of the previous use of iron wire in electrolysis in aneurisms.

	NUMBER OF OBSERVATIONS	CURRENT	AVERAGE COAGULATION TIME	HIGHEST COAGULATION TIME	LOWEST COAGULATION TIME
			minutes	*minutes*	*minutes*
Iron	14	None	8.3	13.5	5.5
Iron	12	1 milliampere	18.7	20	9.0

In every case except two no clot was recorded at the end of twenty minutes. The observation was then discontinued, the cannula examined, and sometimes a small clot was found, sometimes not. In the two cases, the clotting times were 16 and 9 minutes. In the latter case, the two times without current were 5.5 and 7.0 minutes, and a second observation with current ran over twenty minutes.

The average coagulation time when 1 milliampere was passing through iron was increased over the average time with iron and no current by

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at least 125 per cent—how much more cannot be told from the present figures.

An observation of interest in connection with the use of iron and current is the dark coloration of the blood in the cannula about the wire. This color increased and deepened as time ran on until, at the end of the twenty minutes, it was approaching black.

By using certain of the above figures, further comparison may be made between the effects of copper and aluminum.

CURRENT	COPPER	ALUMINUM	PER CENT REDUCTION
	minutes	*minutes*	
None	9.6	5.3	45
1 milliampere	2.9	1.4	52
2 milliamperes	1.9	1.0	47

The average reduction in coagulation time caused by the use of aluminum instead of copper under varied conditions of current flow was 48 per cent.

A matter of considerable importance arises in the kind of clot produced by the metals with and without electricity. In the case of iron the clots under both conditions were like those caused by the presence of a foreign body in blood. In the case of copper normal clots were found when no current was used, but with the current, the tendency was toward a small brittle charred clot, especially with the greater current. In all cases with aluminum the clot was considerably greater in amount, especially with current, and never charred. The consistency was different from that of the normal clot—was more like a gelatin, somewhat friable. The clot did not darken in color.

A single experiment was performed which is capable of quantitative development. Blood was drawn into a well vaselined vessel, so that at the end of forty-five minutes it had not clotted. Into such blood, at different times aluminum, copper and iron wires were plunged, and 2 milliamperes of current passed through them as positive poles. At the end of twenty minutes, practically no clot had formed about the iron wire; a small amount, somewhat charred, about the copper wire; and a considerable amount of gelatinous clot of good color about the aluminum wire. Such results suggest the use of a suitable aluminum wire in the treatment by galvanism of aneurisms that are anatomically favorable.

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SUMMARY

When the wire used in the Cannon-Mendenhall method is aluminum instead of copper, the coagulation time of blood of Belgian hares is reduced 48 per cent.

When the wire is used as a positive pole and 1 milliampere of electricity is passing, the coagulation time is reduced 70 to 74 per cent.

When 2 milliamperes are passing, the coagulation time is reduced 80 to 81 per cent.

When the wire is aluminum instead of copper and 2 milliamperes are flowing the coagulation time is reduced 90 per cent.

When the wire is iron instead of copper or aluminum, the passage of 1 milliampere of current causes an increase in coagulation time of more than 125 per cent—how much more was not determined.

Under most conditions, but especially with current, the clot about the aluminum wire is greater in amount, and more normal in color than the clot about the copper.

My most sincere thanks are due to Dr. W. B. Cannon for his personal interest, help, and inspiration.

My thanks are due, too, to Mr. G. P. Allen who assisted me throughout these experiments with skill.

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VASOMOTOR SUMMATIONS

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In a previous paper (1) the authors have described experiments in which stimulation was applied to afferent paths, singly and simultaneously, to observe the effect upon the blood pressure. It was found that the stimulation of two paths at the same time may be more efficient than the employment of either of these paths alone to elicit vasomotor reactions. The summation of depressor (or excito-dilator) effects was sometimes noted, while with stronger stimuli pressor summations were often obtained. The degree of summation was moderate and it seemed more to be relied on when the nerves chosen for simultaneous stimulation were situated in remote parts of the body.

The experiments now to be reported are upon the vasomotor effects secured by stimulating two nerve-paths of which one was always the central vagus. This trunk, in the cat, usually contains the fibers which have the depressor property in the highest degree—those which are regarded as constituting *the* depressor nerve. We have published a study of the vasomotor responses obtainable from the central end of the vagus with measured intensities of stimulation (2). It has been our impression that two thresholds are crossed as the stimuli applied to the vagus are progressively increased in power: the first is the threshold of the 'mild' (excito-dilator) reaction while the second ushers in the true depressor phenomenon. By this we understand a strong inhibition of the vasoconstrictor center. The mild reaction entails a lowering of blood pressure not often exceeding 15 per cent, with a tendency to recovery during the continuance of the stimulation. The full depressor response is manifested by a fall of pressure amounting to 30 per cent or more and there is little sign of rallying even during several minutes' stimulation.

When a peripheral nerve other than the vagus is subjected to increasing stimulation we have again evidence that two thresholds are successively surmounted (Martin and Lacey, (3)). Weak stimuli produce a fall of pressure approximately similar to that secured from the vagus

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while at about the same level at which the vagus begins to give the full depressor response most other nerves begin to evoke a rise of pressure. The simplest explanation has seemed to be that the lower threshold in both cases is that of the vasodilator mechanism while the higher one is that of the vasoconstrictor center. It is assumed that this center may be influenced in the direction either of inhibition or excitation and that the requisite stimuli for producing the two effects are of the same order of intensity.

Procedure. The cats were anesthetized with urethane, ether being used occasionally to deepen the narcosis. This was seldom necessary. Both vagi were cut. Stimulation was applied to the nerves by means of platinum electrodes in glass tubes, a modification of the Sherrington electrodes devised by Martin. For the simultaneous stimulation of two nerves two calibrated induction coils were used. The secondary circuits were independent while the primary coils were in series upon one circuit. This common primary circuit was interrupted by Martin's mercury key (4) actuated by a crank on a motor-driven shaft. The primary current was sometimes of 0.1 ampere, sometimes of 0.2 or 0.3. The rating of the shocks in the Z-units of Martin (5) could be found at any time by the use of calibration tables. The rate of interruption was about 8 to 12 per second. Both make and break shocks were allowed to take effect, polarization being thus minimized. The nerve most often used with the vagus was the peroneal.

Results. The summations with which we have had to deal have been sometimes algebraic and sometimes arithmetical. That is to say, we have sometimes had to do with antagonisms and sometimes with reinforcements. The reinforcements present the simpler condition and may first be discussed. At one time or another we have seen the realization of almost every theoretical possibility. For example, we have the case in which stimulation of the vagus with a certain current-strength leads to a mild fall of pressure while stimulation of the peroneal with an appropriate current causes a fall of the same order. Under such conditions we have found that the fall of pressure following simultaneous stimulation of the two nerves with the same current as before may be just about the sum of the two separate depressions.

An example of this ideal depressor summation may be given. A stimulus rated at 195Z applied to the vagus gave a drop of 8 per cent (a mild type of reaction). A stimulus rated at 406Z applied to the peroneal reduced the pressure by 12.5 per cent. When the same stimulation as before was given both to the vagus and to the peroneal at one time the resulting fall of pressure was 20.6 per cent. The combination

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has a magnitude hardly ever reached with nerves other than the vagus and is in the border region between the mild and the profound reactions as exhibited with that nerve. In another case the following summation was recorded:

Stimulus to peroneal 457Z, drop	10 per cent
Stimulus to vagus 450Z, drop	22 per cent
Both together, same stimuli, drop	29 per cent

Here the united effect falls short of the sum of the two.

In a few instances the depression produced by stimulating the two nerves at once was in excess of the sum of the separate pressure reductions. For example, we have the following:

Stimulus to peroneal 406Z, drop	12.5 per cent
Stimulus to vagus 195Z, drop	9.0 per cent
Both together, same stimuli, drop	28.0 per cent

Here the vagus effect by itself is clearly of the mild type while the combined approaches the full depressor character.

A tracing here reproduced (fig. 1) shows a depressor summation on a scale of unusual magnitude. The animal was an exceptional one in that stimulation of the sciatic nerve gave pronounced lowering of the blood pressure and that no reversal of effect (pressor reaction) was obtained from this nerve with the greatest intensity of stimulation. In the record, 36 is the response to simultaneous stimulation of the vagus (1700Z) and the sciatic (2460Z). The fall is 42.5 per cent. No. 39 is a check on 36; the fall is 39 per cent. No. 37 is the response to sciatic stimulation alone (2460Z); the pressure falls 29 per cent. At 38 the vagus alone was stimulated (1700Z) and the fall was 20 per cent.

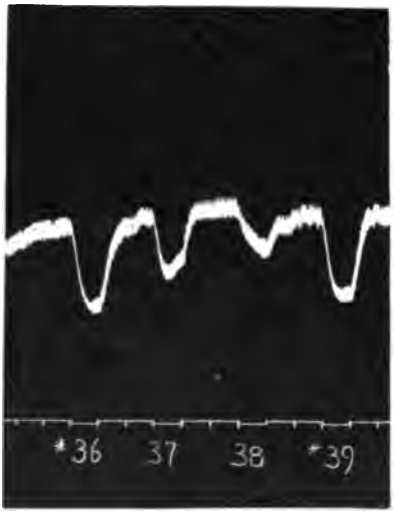


Fig. 1.

We must now consider the summation effects obtainable when the separate effect from the leg nerve is a rise of pressure. There are two distinct opportunities for investigation: the pressor reaction may be

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matched against the mild lowering of pressure secured by weak excitation of the vagus or it may be attempted when the vagus stimulation is strong enough to induce, by itself, the typical depressor response. In the former combination we have found that the fall of pressure can be converted to a rise.

Bayliss (6) long ago (1893) reported the possibility of antagonizing the depressor in the rabbit by strong stimulation of another afferent nerve. His study was qualitative in character but he states that with

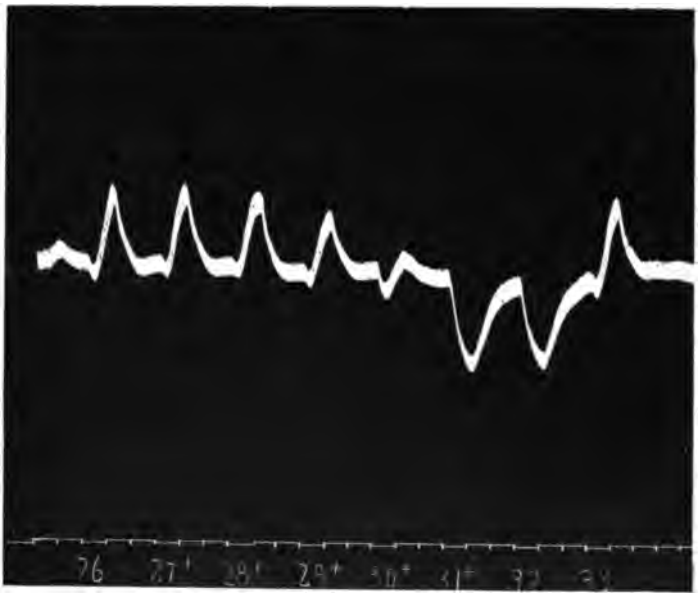


Fig. 2.

properly chosen strengths of stimulation he found it possible to obtain a neutralization of effects and to hold the blood pressure undisturbed at its original level.

We may say with equal truth that a rise of pressure can be converted to a fall. Attention may be called to the following series of results, obtained in trials in which a constant peroneal (pressor) stimulation was opposed by vagus stimulation of increasing intensity. (The tracing for the second part (nos. 26–33) is reproduced, figure 2.) The tabulation is below.

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Peroneal, 990Z, vagus, 223Z, pressure rises..... 34.5 per cent
Peroneal, 990Z, vagus, 265Z, pressure rises..... 32.0 per cent
Peroneal, 990Z, vagus, 400Z, pressure rises..... 14.8 per cent
Peroneal, 990Z, vagus, 554Z, pressure *falls*..... 21.0 per cent

Again:

(26) Peroneal, 670Z, pressure rises..... 24.0 per cent
(27) Peroneal, 670Z, vagus, 126Z, pressure rises..... 25.0 per cent
(28) Peroneal, 670Z, vagus, 180Z, pressure rises..... 24.0 per cent
(29) Peroneal, 670Z, vagus, 223Z, pressure rises..... 19.5 per cent
(30) Peroneal, 670Z, vagus, 265Z, pressure *falls*..... 7.0 per cent
(31) Peroneal, 670Z, vagus, 400Z, pressure *falls*..... 32.5 per cent
(32) Vagus alone, 400Z, pressure *falls*..... 29.6 per cent
(33) Peroneal alone, 670Z, pressure *rises*..... 30.0 per cent

Here we see the vagus asserting its full power to depress the blood pressure in spite of a simultaneous application of a pressor stimulus which by itself proved highly effective at the close of the experiment.

We have observed repeatedly that if we calculate the ratio between the stimulus applied to the vagus and that applied to the leg nerve when the result is a neutralization of one reaction by the other, the value obtained is roughly constant. For instance: a stimulus to the vagus rated as 400Z offset the influence of a stimulus to the peroneal rated as 990Z. The ratio $\frac{990}{400}$ is equal to 2.48. Later in the same experiment a vagus stimulus described as 265Z counterbalanced 670Z on the peroneal. The ratio $\frac{670}{265}$ is equal to 2.53. Ratios from another experiment may be given in tabular form:

1300Z on the peroneal balances 1100Z on the vagus..... Ratio, 1.18
940Z on the peroneal balances 960Z on the vagus..... Ratio, 0.98
750Z on the peroneal balances 770Z on the vagus..... Ratio, 0.98
554Z on the peroneal balances 558Z on the vagus..... Ratio, 0.99

The fact that these ratios are close to unity has no significance. We have not taken into account the unequal resistances of the two nerves. For the same reason we cannot institute comparisons between data secured in different experiments. Neither is it strange that in any one experiment the constancy of the ratio is eventually lost, for a decline in the condition of the two nerves and their connections is to be expected. The one which is first to show a rising threshold will naturally cease to interact with the other upon the original footing. The signifi-

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cant thing is that even under temporary and favoring conditions the ratio should be demonstrable at all.

It remains for us to speak of the results observed when we have attempted to convert a full depressor reaction into a rise of pressure. When we apply to the vagus a stimulus strong enough to give a maximal lowering at both the beginning and the conclusion of a series of trials we find that we can lessen the vagus effect and at last make it insignificant by the simultaneous application of powerful pressor stimulation. The interesting fact is that we cannot transform it into a rise. Consider the following figures:

Vagus by itself, 558Z, pressure falls..... 45 per cent
Peroneal, 125Z, and vagus, 558Z, pressure falls..... 43 per cent
Peroneal, 265Z, and vagus, 558Z, pressure falls..... 41 per cent
Peroneal, 554Z, and vagus, 558Z, pressure falls..... 35 per cent
Peroneal, 940Z, and vagus, 558Z, pressure falls..... 30 per cent
(There was a recovery within the period of stimulation until the final level was only 12.8 per cent below the initial.)
Peroneal, 1300Z, and vagus, 558Z, pressure falls..... 16.5 per cent
(There was a recovery of the initial level but no rise above it within the period of stimulation.)
Peroneal, 1600Z, and vagus, 558Z, pressure falls..... 8 per cent
(Again there was recovery of the initial level but no rise above it.)
Peroneal, 1800Z, and vagus, 558Z, pressure practically unchanged
Peroneal, 1800Z, without the vagus, pressure *rises*..... 24 per cent

Figure 3 shows the type of record just described. At 38 the vagus alone is stimulated (430Z). The fall is 36 per cent. In each of the following trials, through 45, the same strength of stimulation is applied to the vagus. Simultaneous stimulation of the peroneal is as follows: No. 39,—125Z; No. 40,—265Z; No. 41,—554Z; No. 42,—940Z; No. 43,—1300Z; No. 44,—1600Z; No. 45,—1800Z. The depression produced by the vagus is annulled by these strong peroneal stimulations but there is no appearance of an actual elevation. No. 46 is the pressor response to peroneal stimulation (1300Z); the rise is 20 per cent. (It is to be noted that the pressure falls *after* each of the combined stimulations, 41–45. The attention for the present is to be fixed on the period of stimulation and not on the after effect.)

It is manifest from this and other cases that the strong stimulation of the leg nerve simultaneously with the vagus limits the duration of the depressor effect. It may entirely counteract that effect also but we have not seen the pressor dominant over the depressor in any instance which could be regarded as trustworthy. In rare cases where

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such a reversal has seemed to appear a diminishing irritability of the vagus or its central connections was found to have been present.

After effects of stimulation. Stimulation was usually applied for periods of thirty seconds. In most cases the maximum change of blood pressure which can be looked for is recorded within this interval. But attention is to be called to an interesting exception. This is noted when strong stimulation of the vagus is balanced by strong stimulation of another nerve. The reaction during the period of excitation may be a small fall of pressure, as already indicated or in some cases a preliminary larger fall, followed within the period of stimulation by re-

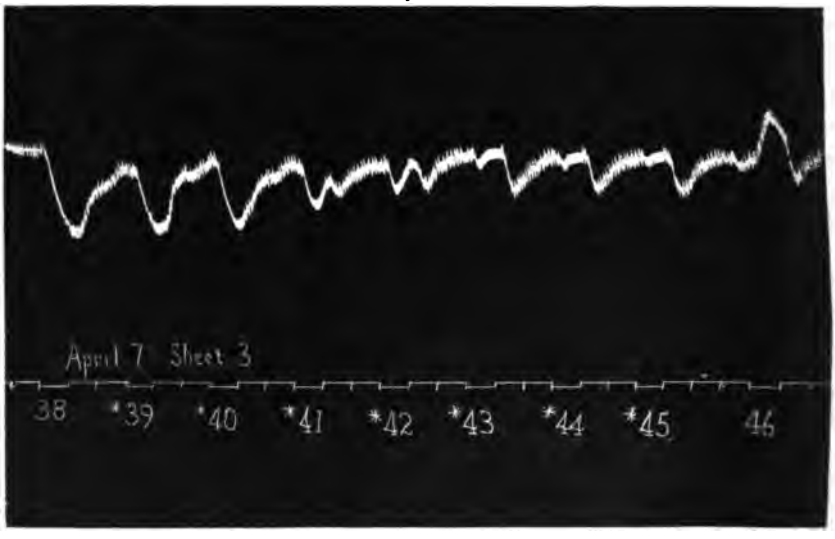


Fig. 3.

covery. When stimulation is discontinued in such instances it is commonly observed that the pressure drops considerably more than it had previously. Within another thirty seconds it returns to an average level. In other cases where the effect during stimulation is a small or moderate rise a reversal may follow, the pressure dropping far below the average value.

DISCUSSION

Depressor Summation. We have described above, experiments in which we applied to the central vagus and another afferent nerve, simultaneous stimulations of such intensity as in either case alone to

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give mild (excito-dilator) depressions. In comparison with similar experiments in which two afferent nerves, exclusive of the vagus, were employed, the striking difference was the much greater degree of depressor summation obtainable with the inclusion of the vagus. Evidently the vagus is not precisely equivalent to other sensory nerves; or, to put the point in another way, the mild depression observed when the vagus is moderately stimulated does not correspond in all respects with the mild depressions obtained under similar conditions from excitation of other nerves. The difference that at once suggests itself is that in the background of the ordinary sensory nerve are fibers whose excitation will antagonize the depressor response, whereas the corresponding fibers of the vagus instead of antagonizing will accentuate it. The fact that summation is possible shows that the two nerves together act more potently on the central mechanism than either alone. Where both contain pressor fibers, it is conceivable that, although these fibers are not clearly competent, their combined action may nevertheless limit depressor summation to the moderate amount previously reported by us.

We have thought of the pressor mechanism as having a much higher threshold than the depressor; we need to bear in mind, however, that the threshold of reversal from depresssor to pressor reaction is probably higher than the actual threshold of the pressor mechanism, for before a depressor reaction can be changed to a pressor in the presence of continuous depressor excitation a degree of activity considerably above the threshold must have been attained. There would seem to be no reason why with stimuli approaching the reversal point we might not have enough pressor activity to serve as a basis for summation, even though not enough to counteract the depressor reaction. With the vagus forming one of the nerves used in evoking summed responses the depressor responses are summated but there is no corresponding summation of pressor influences. Rather, any that may be present in the ordinary nerve excited are neutralized by the vagus. Full scope is therefore given for the development of marked depressor reactions. The case cited by us in figure 1, where the pressor influence of the sciatic nerve was apparently in abeyance, illustrates the same point more emphatically. In this instance, because there was no pressor tendency, much stronger stimuli could be applied than was usually possible in these experiments (1). The depressor summations obtained were correspondingly more pronounced.

Summation of antagonistic influences. When afferent nerves other

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than the vagus are strongly excited pressor reactions are, as a rule, to be obtained. We have shown that these can be successfully antagonized by suitable vagus excitations. Scrutiny of the conditions of antagonism brings out strongly the remarkable constancy of operation of what is thought of as an exceedingly complex mechanism. W. T. Porter (1910) (7) has previously emphasized this constancy of vasomotor responses. Under ordinary experimental conditions the mechanism must be pictured as in an equilibrium which includes a fairly steady vigor of discharge of vasomotor impulses; these establishing the condition that we describe as vasomotor tone. Afferent stimulation disturbs this equilibrium; either upward or downward according to the nature of the stimuli. Our experiments show that a disturbance upward can be neutralized by a suitable depressor influence, and furthermore, that the strength of depressor stimulation necessary for its neutralization bears, in any experiment, a fixed ratio to the strength of the excitation by which the elevation of tone was brought about. In this feature we emphasize, even more strikingly than does Sherrington (8) in his experiments on antagonism of skeletal muscle reflexes, the "algebraic summation" which characterizes the reaction of the central nervous system to opposing stimulations.

A feature of the antagonism which is possibly significant is that in general the depressor influence is more potent than the pressor. This statement may appear inconsistent with our previous emphasis on the mathematical relationship existing between the two influences. What we desire to point out, however, is this: although the amount of depressor stimulation necessary to neutralize any given pressor influence is in fixed proportion to the pressor influence, if a stronger depressor stimulus be applied than that which just balances the pressor the resulting depression is disproportionately great. This we have seen repeatedly. An instance appears in figure 2, where a pressor stimulus of 670Z which by itself caused a substantial rise of pressure (nos. 26 and 33) and required for neutralization a vagus excitation of 265Z (no. 30) was apparently wholly ineffective when the vagus stimulus was increased to 400Z (no. 31). A parallel case in which the antagonistic factor was asphyxia has been reported by Asher (1906) (9).

Another line of evidence which suggests the prepotency of the depressor is exemplified in figure 3, in which a very considerable increase in pressor stimulation strength beyond that required to neutralize a strong depressor effect did not carry the blood pressure above the level of neutralization. This latter observation, in fact, shows clearly that

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the blood pressure level is not determined altogether by the algebraic sum of all the afferent impulses calculated to modify it. The vigor of discharge which determines vasomotor tone may be restored by strong pressor stimulation when strong depressor excitation is tending to lower it, but in the presence of such depressor excitation it is difficult if not impossible to force a vigor of discharge greater than that of normal vasomotor tone. If an illustration may be ventured, the vasomotor tone may be compared with the level of water in a tank which has a supply-pipe and an overflow. Increasing the supply (pressor stimulation) may force the level above that of the waste-way. Depressor stimulation is analogous to draining the water by opening an outlet at a lower level than that of the ordinary overflow. When this extraordinary draining of the water is in progress it is easy to imagine that driving the feed-pump to its limit may restore the normal height of the surface but cannot raise it higher because of the double escape which becomes available (fig. 4). The final explanation of this phenomenon must wait for knowledge beyond that which we now possess of the detailed functioning of the central nervous system.

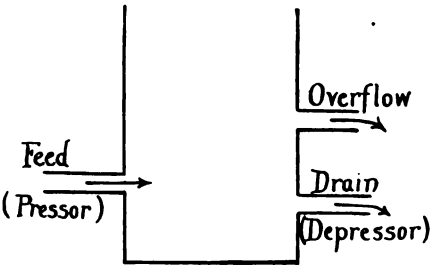


Fig. 4.

The depressor after effect. Our observation that the after effect of combined strong pressor and depressor stimulation is a manifestation of persistent depressor influence appears at first view to be at variance with the observations of Baxt (1875) (10) on the heart and of v. Frey (1876) (11) on the submaxillary gland, in which opposing peripheral influences were excited simultaneously, and in which the *stimulation* phase was the one to appear after cessation of excitation. Asher, in the paper cited above (9), reports that the asphyxial pressor influence is the one to show itself when asphyxia and depressor excitation are withdrawn simultaneously. In the single protocol given by Asher (loc. cit., p. 94) the asphyxial rise is recorded as reaching its height two seconds after the release of the tracheal clamp, an interval scarcely long enough to have permitted adequate oxygenation of the vasomotor center. It is possible, therefore, that there was not in that case a true after effect, but an actual persistence of asphyxial excitation, after the withdrawal of the depressor stimulation. A well-known feature of the

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response of the vasomotor mechanism to strong depressor stimulation is the sluggishness of the return to the former level. This is well illustrated in figure 3, no. 38. Such a curve as no. 43 in the same figure suggests that the depressor influence although not able to bring about the usual change in blood pressure in the presence of strong pressor excitation, is nevertheless acting on the mechanism in precisely its usual fashion. Immediately upon the withdrawal of the pressor influence the blood pressure takes the position it would have had at the same moment had there been no pressor factor. The return to the normal level is of the same degree of sluggishness as after depressor stimulation alone. This view of the nature of the inhibition of the vasomotor center by depressor excitation places it in Heidenhain's (12) second class of inhibitory actions, the class in which inhibition and augmentation act on different parts of the affected mechanism. Asher (loc. cit., p. 96) takes this same position.

On the other hand, our observations discussed above, on the "algebraic summation" of pressor and depressor influences, would appear to place the vasomotor center in harmony with the center for skeletal muscle reflexes, in which, as stated, Sherrington has shown the principle of algebraic summation to obtain, and in which on that account, he postulates the inhibition to be of Heidenhain's *first* class, a direct opposition to excitatory influences. If our interpretation of the inhibitory after effect is sound, we shall be led to the conclusion that exciting and inhibitory influences may act simultaneously upon a mechanism in such a manner that each exerts its normal effect in the presence of, but masked by, the other, and yet be so related as to show very exact algebraic summation.

SUMMARY

1. Depressor (excito-dilator) summation is more pronounced when one of the afferent nerves excited is the vago-depressor trunk than when the summation is secured by stimulation of two afferent nerves other than the vagus.

The suggestion is offered that the presence in afferent nerve-trunks generally of pressor fibers may limit the degree of excito-dilator summation obtainable through excitation of such trunks. The corresponding fibers in the vagus are depressor, and tend to favor, rather than to limit excito-dilator summation.

2. The antagonism between pressor and depressor influences is shown to have the character of algebraic summation when the intensity of

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depressor stimulations necessary to neutralize a series of pressor influences is taken as the criterion.

3. Depressor stimuli stronger than just sufficient to overcome concurrent pressor influences have an effect disproportionate to their mathematical superiority.

4. Strong depressor stimuli can be neutralized by strong pressor excitation, but it is difficult, if not impossible, to force blood pressure above the normal level thereby.

5. The after effect of pressor-depressor summation is typically a transient fall of blood pressure. The appearance of the curve suggests that the depressor influence is masked but not destroyed by concurrent pressor excitation.

6. The nature of the after effect indicates that depressor and pressor influences act upon different parts of the central mechanism. The algebraic summation has been formerly interpreted to mean opposing action at a common point. The suggestion is made that action at different points may be consistent with algebraic summations.

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THE MOVEMENTS OF THE MITRAL CUSPS IN RELATION TO THE CARDIAC CYCLE

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I. INTRODUCTION

The generally accepted conception regarding the movements of the auricular-ventricular valves in the beating heart, as stated in several modern text books of physiology, may be summarized as follows: When the auricle contracts it forces a small quantity of blood into the relaxed ventricle. This raises the intra-ventricular pressure a trifle, and by forming eddies behind the valve-cusps causes their approximation or partial closure. Thereupon follows ventricular systole with the immediate rise of intra-ventricular pressure. When the intra-ventricular exceeds but slightly the intra-auricular pressure, complete closure of the auriculo-ventricular valves occurs, and regurgitation is prevented.

The accuracy of this conception has recently been reinvestigated by Henderson and Johnson (1) who mounted in a glass jar the excised valves of ox hearts, in such a way that the natural connections of the valves were undisturbed. The action of these valves could be clearly observed when pressure changes, simulating those within the heart, were artificially produced from either the auricular or the ventricular side. The chief conclusions drawn from this study were:

1. Auricular systole forces a jet of blood through the auriculo-ventricular openings. When this jet breaks at the end of auricular systole, a zone of negative pressure is formed between the cusps. This unrolls the edges of the valves, approximating them in complete and final closure just previous to ventricular systole. It is this method of closure which, according to these investigators, prevents regurgitation in the normally beating heart.

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2. When ventricular systole is not preceded by an auricular contraction in the cycle (as, for example, in auricular fibrillation), the rise of intra-ventricular pressure closes the valves. This type of closure is, however, abnormal in that the valves do not unroll, but close by a "hinge movement" which is necessarily accompanied by a slight regurgitation.

If these conceptions of Henderson and Johnson be correct, we must revise our current ideas not only of the mechanism of valve movement, but also of the temporal relation of valve closure to auricular and ventricular systoles. For example, it is necessary to assume that the valves close at the end of auricular systole and before ventricular contraction begins. If this be the case, it may offer an explanation as Lewis has suggested, of the pre-systolic sound heard whenever the a-v interval is long (2). Furthermore, if their view is correct that ventricular systole itself is not directly responsible for the valve closure, we may be compelled to modify our views as to the dual origin of the first heart sound.

From a physiological as well as a clinical standpoint, therefore, it is desirable to again investigate the question as to the movements of the valves within the beating heart, and the precise relation of their movements to the cardiac cycle.

II. METHOD

Doubtless the most accurate and reliable method of studying this question would be to attach directly to the valves of the intact heart freely movable threads or hairs, and transmit their vibrations directly to recording levers capable of accurately following the slightest valve oscillation. This, so far, has not proven feasible. After considerable preliminary experimentation, however, it was found possible to study the valve action of the perfused cat's heart in this way. The description of the method naturally divides itself into a consideration of (1) the preparation of the heart and (2) the method of recording.

Preparation of the heart. The chest of an etherized cat is opened and the animal allowed to die by asphyxia. The cat heart is chosen because of anatomical advantages, and this method of causing death is used because comparatively little harm is done the heart. When the heart has ceased beating, it is removed from the chest, care being exercised to leave enough of the venae cavae and aorta attached. As soon as possible, a T-cannula (*P*) is fastened into the aorta (*O*),

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and warmed Locke's solution is perfused through the heart under a suitably gauged pressure (fig. 1). A T-cannula is used so that one limb, not tied into the aorta, transmits Locke's solution from the main reservoir, the other, fitted with a thin-walled, partially clamped, rubber tube, serves as a continuation of the elastic aorta (*Q*). Thus the ventricle contracts against an aortic pressure which varies during each cycle as in the intact heart.

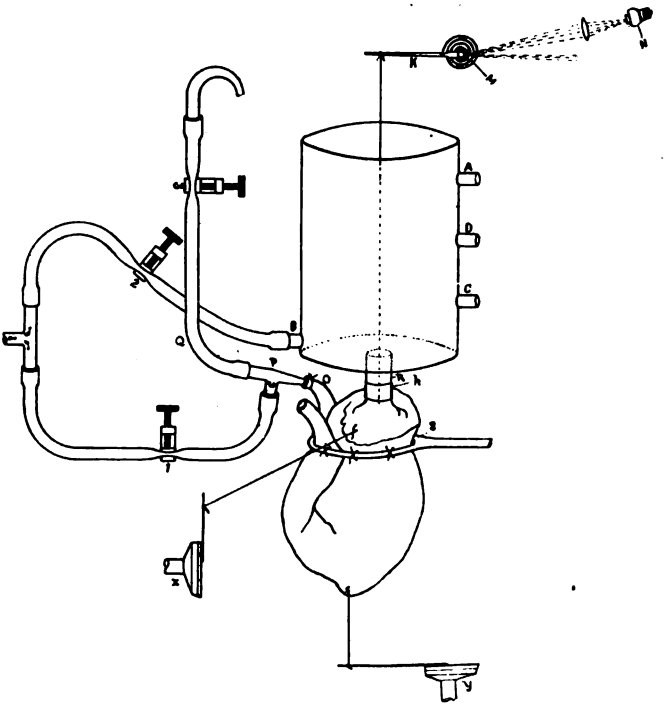


Fig. 1. Diagram of apparatus—description in text.

Next, an opening about 3 mm. in diameter is cut just posterior to the left auricular appendage, and the pulmonary veins ligated. With a small, curved needle, possessing an eye near its point, a human hair is passed downward, then upward through the medial or septal cusp of the mitral valve near its free margin, and there securely tied. Only a very small portion of the cusp is caught in the ligature. After many trials it was possible to catch the same point of the cusp in each experiment. This was verified by subsequent examination. The short

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end of the hair is next cut off close to the cusp to avoid extraneous contacts.

As a means of maintaining an auricular pressure which, at the same time, could be modified at will, the glass reservoir-cylinder shown in figure 1 was devised. Around the bottom exit (*R*), adhesive plaster is wrapped. Between this and the opening made into the left auricle, an impervious suture is made, the hair attached to the valve having been previously led upward through the opening. Through a low lateral opening (*B*) of the reservoir, perfusing fluid enters, and by stopping either of the lateral exits (*C*, *D*, or *A*), the auricular pressure may be varied in steps of 20 mm. of Locke's solution. In this way pressures varying between 45 and 85 mm. of fluid were maintained.

Ligatures are placed at suitable intervals through the musculature of the heart at the level of the auriculo-ventricular ring. By these the heart is anchored to a rigid and neatly fitted ring of metal (*S*) through which it is suspended. Thus the auriculo-ventricular ring does not change its position while the heart beats. This procedure is necessary in order to prevent movements of the cardiac musculature from being registered through the thread attached to the cusp. Care must be taken, of course, not to exert such tension upon these threads as to cause any relative valvular insufficiency, nor may any but very small blood vessels be caught within the ligatures.

Method of recording. The contractions of the left auricle and left ventricle are recorded optically on a moving bromide film, by connecting them through threads to the straw levers of recording segment tambours (*x-y*). These communicate by rubber tubes with Frank's recording segment capsules (*N*).[1]

The movements of the septal cusp of the mitral valve are communicated by the hair to a light lever (*K*), rotating about a fixed axis and held up by a tiny coil very much as in Frank's recording manometer. The rotation of this axis is recorded by reflecting a beam of light into the tiny mirror (*M*) fastened directly to this axis.

The common source of light for this mirror as well as those of the segment capsules was Frank's arrangement of a Nernst filament, whereby three aligned beams of light are projected, and each focused upon its mirror. The mirrors in their turn reflect beams of light through the slit of a photokymograph and so record upon moving bromide paper.

[1] For a description of these capsules see Wiggers: Journ. Amer. Med. Assn., 1915, lxiv, 1485.

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III. VALVE MOVEMENTS WHEN AURICLES OR VENTRICLES BEAT ALONE

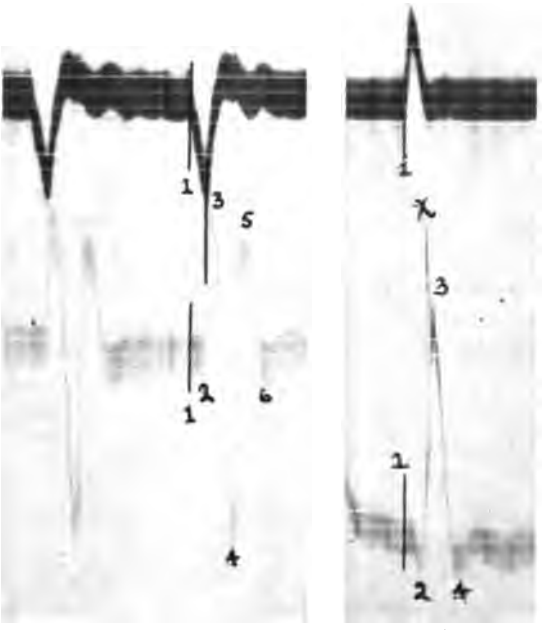
To facilitate the analysis of records of normally beating hearts, it seems desirable to first study the movements of the valves when the auricles and ventricles beat separately. The cases so analysed represent in part those instances in the perfusion experiments in which the auricles or ventricles beat alone. Such occasions are always found in any series of perfusion experiments, but here only records were considered in which there was active beating of the auricles without any signs of ventricular movement, or vice versa. Furthermore, it was easily possible at the end of the experiments, to fibrillate the auricles or ventricles with a tetanizing electric current and in this way study their separate effects.

The movements of the valves *when the auricles beat alone* are illustrated in the two segments of figure 2. Two types of movement were recorded. As shown in curve A a short interval after auricular systole begins, but before its termination, an upward oscillation of the cusps occurs (cf. points *1* and *2*). This is followed during relaxation of the auricle by a downward movement *3–4* and an after-vibration of the valves (*4*, *5*, and *6*). The amplitude of the initial and after-vibrations is somewhat dependent upon the intra-auricular pressure. The after-oscillation is not at all typical of the cusp movement in the majority of experiments, and, apparently, only occurred when the ventricles were extremely flaccid. The usual form of movement is a single vibration, during auricular contraction and relaxation, as shown in figure 2*B*. This is preceded in most instances by a very slight downward movement (*1*, *2*) synchronous with the beginning of auricular systole. It is difficult to say whether the sharp, superimposed vibration of shorter period (*X*), only occasionally present, is due to valve vibration or is of instrumental origin since its period corresponds suspiciously well with that of the lever system used.

In none of the curves was there found any evidence that the movement toward closure, caused by auricular activity, was anything but a transient oscillation, absolutely incapable of being the efficient valve closure mechanism, not only because the valves were not maintained in a closed state, but also because effective apposition of the cusps did not occur. This could be observed ocularly.

When the ventricles beat alone the curve of valve closure is entirely different as is shown in figure 3. At the onset of systole (*1*) the valves move upward quickly, and are completely closed an instant before the

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A *B*

Fig. 2. Two segments of records showing oscillations of mitral cusps when auricles beat alone. *A*, Upper curve, auricular myogram (systole downstroke)—lower curve valve movements. Intraauricular pressure, 60 mm. Locke's solution. Position of points indicated by line 1. *B*, Upper curve, auricular myogram (systole upstroke)—lower curve, valve movements. Rise of curve indicates movement of valves auricleward. Intraauricular pressure, 75 mm. Locke's solution.

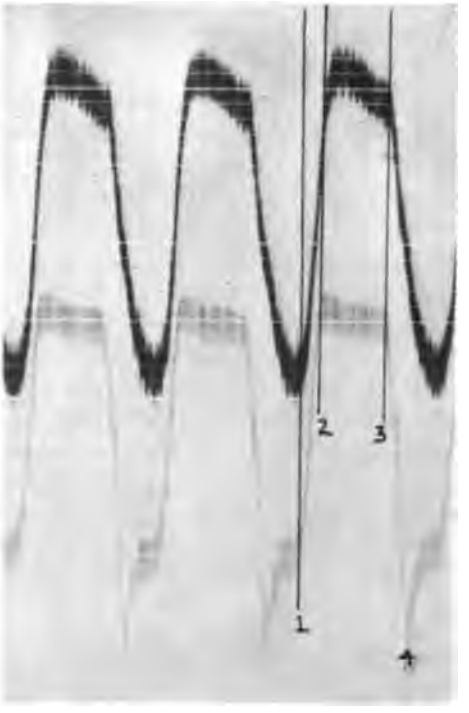


Fig. 3. Upper curve ventricular systole (rise) begins at *1* and terminates at *3*. Lower curve, movements of mitral cusp, upstroke signifies auricleward movement.

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maximum of ventricular contraction as recorded from the apex (*2*). The valves remain closed throughout systole, giving the curve a plateau form (*2–3*). Synchronous with relaxation (*3*) the valves open quickly, moving down to a lower position than they occupied before the onset of systole (*4*). From this point they are slowly buoyed upward as blood flows into the ventricle from the auricle during the remainder of diastole. Vibrations such as might account for the first sound were never found to be superimposed on the curve of valve closure when the ventricle beat without the auricle.

IV. VALVE MOVEMENTS DURING THE NORMAL SEQUENCE

When auricles and ventricles beat in normal sequence the curves show essentially the combined types of movement described separately above. Figure 4 shows the curve from an experiment where a great magnification of valve movement occurred. Auricular systole begins at point *A*, and is accompanied by a slight downward movement of the valve-cusp record. Just before the end of auricular systole the mitral valve rises sharply (*B*) and returns promptly at the onset of auricular diastole (*C*). The valve does not fall back as far as before, probably because of increased intra-ventricular pressure. Now comes ventricular systole (*D*) when the mitral cusps quickly rise to effective closure and remain in this condition until ventricular relaxation begins, when they rapidly open. A rebound often occurs as is shown in figure 4 by oscillation (*E*). No other diastolic changes occur when, as in this instance, the heart rate is rapid and the period of diastasis therefore practically absent.

Figure 5 illustrates even better the nature of the cusp movements since less magnification was used. In this case the heart rate was also slower, and a period of diastasis (*G–A*) occurred. The valve movements alone are here reproduced. If it is borne in mind that every upstroke of the curve indicates an upward, and every down stroke a downward movement of the valve cusps, we can readily obtain a vivid mental picture of the exact movements undergone by the mitral cusps in the normal cycle. It is clear that auricular systole and ventricular systole each produce in successive order an upward or closure movement of the valves. With the onset of auricular systole, probably due to the active impingement of blood upon the mitral flaps, a slight downward movement (*AB*) usually takes place. Then, near the end of auricular systole, the valves move upward toward the position of clos-

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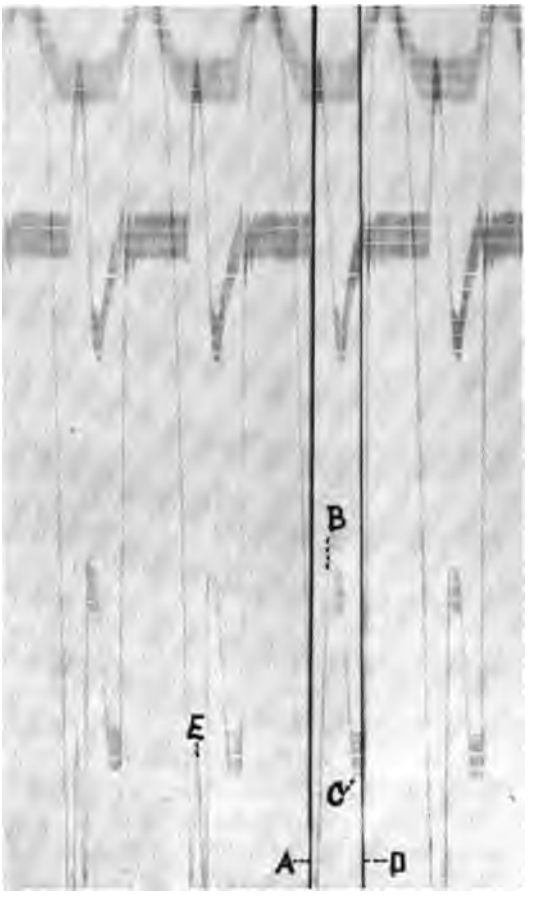


Fig. 4. Uppermost curve, ventricular systole (upstroke), middle curve, auricular systole (upstroke)—lower record, movements of mitral cusps. Speed of paper 28 mm. per second.

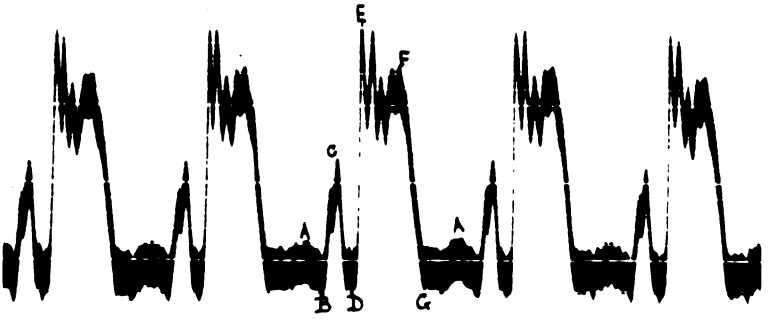


Fig. 5. Curve showing movements of mitral valves when normal sequence and a long As-Vs interval obtains. *AC*, auricular closure of valves, *DE*, ventricular closure.

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ure (*C*). This closure is, however, only temporary and not complete, the valves returning promptly to their former open position (*D*). At the onset of ventricular systole, the valves are closed completely (*E*), and remain so during systole (*EF*).[2] Having returned to a point in early diastole lower than they occupied previous to the onset of ventricular systole (*G*), the cusps gradually rise auricleward during the period of diastasis (*GA*).

V. RELATION OF VALVE MOVEMENTS TO LENGTH OF As-Vs INTERVAL

In the curves shown in figures 4 and 5 the As-Vs interval often measured 0.288 seconds, a period considerably longer than is assumed to exist in the human heart from electrocardiographic studies (0.13–0.18 sec.). This long interval made these records especially valuable for analysing the several factors involved in valve closure. The question arises, however, whether two closure movements of the valves can occur in every cardiac cycle when the As-Vs interval is shorter. It is clear as shown diagramatically in figure 6, that when the As-Vs interval is progressively shortened, the curve representing the ventricular closure of the valves first follows the auricular at a shorter interval (fig. 6 *B*), then becomes supported on the auricular curve (fig. 6 *C*), and finally becomes continuous with it (fig. 6 D). For descriptive purposes these forms of closure may be designated as types *A*, *B*, *C*, and *D*.

In interpreting the valve movements of the intact heart, therefore, it is important to determine (1) how short the As-Vs interval must become before the ventricular closure begins to be supported on the auricular, (type *C*), and (2) how short the interval must become before the influences of auricular and ventricular systole blend in a single closure movement (type D).

Computations from a number of different experiments show that auricular systole precedes the auricular cusp vibration by about 0.084 seconds, and that the auricular closure of the valves, itself requires on an average 0.063 seconds (fig. 4, *AB*). It is apparent from these results that unless the As-Vs interval is greater than the sum of these figures (0.084 seconds + 0.063 seconds, i.e., 0.147 seconds), the valves have not time to reopen before ventricular systole, and the closure movement

[2] It is not possible at present to interpret the significance of the smaller oscillations superimposed upon the main curve—whether they are vibrations of the closed valves, responsible in part for the first heart sound will be investigated in a future research.

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takes the form of type D (fig. 6). This closure is initiated by auricular systole, but is completed and maintained by ventricular contraction. As soon as the As-Vs interval becomes only a trifle greater, however, the valves, after auricular systole, tend to open to a slight degree, and, as this interval becomes progressively longer, the valves open more and more before ventricular closure (type C). Since auricular systole precedes the onset of auricular closure by 0.084 seconds, and the closure plus opening requires 0.188 seconds, it is evident that complete opening does not occur unless the entire As-Vs interval equals at least the sum of these figures or 0.272 seconds.

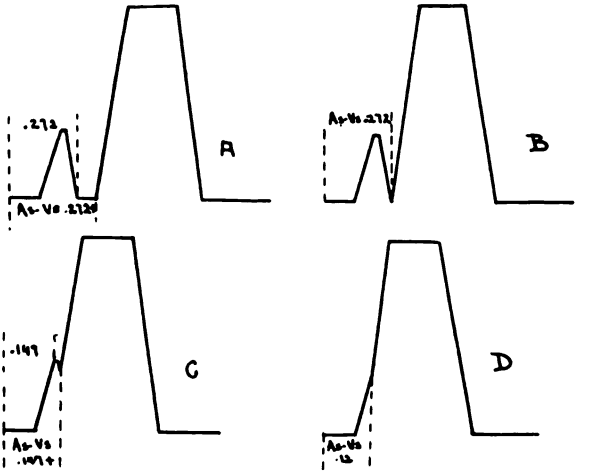


Fig. 6. Schematic drawing showing influence of the length of the As-Vs interval on the valve movements. Types *A* and *B* occur when the As-Vs interval exceeds 0.272 seconds. Type C occurs when the interval ranges between 0.147 and 0.272 seconds. Type *D* occurs when the interval is less than 0.147 seconds.

VI. SUMMARY OF RESULTS

1. By making a direct connection between the septal cusp of the mitral valve and a lever delicate enough to register its slightest oscillations, it was possible to record optically the movements of the mitral cusps as they occurred in the cycle of a perfused cat's heart.

2. When the As-Vs interval averaged 0.272 seconds or more (fig. 6, *A*, *B*), the movements of the mitral cusps during each phase of the normal cycle were as follows:

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a. A short period after the onset of auricular systole the cusps move ventricleward slightly. Toward the end of auricular systole they move auricleward quickly and markedly, but not to a position of complete closure.

b. At the onset of auricular diastole the cusps quickly move ventricleward, the rapidity depending upon the existing intra auricular pressure. When ventricular tonus is low, intra-ventricular pressure is low, and when this obtains there is a rebound of the cusps from the ventricular walls. The valves remain open until ventricular systole begins.

c. At the onset of ventricular systole the cusps immediately move upward to a condition of complete closure, and remain so until ventricular relaxation begins.

d. During active relaxation of the ventricles the cusps move downward to a lower position than they occupied at the beginning of systole. From this position they gradually float upward during diastasis.

3. The sequence of movements above described also occurs when the As-Vs interval ranges from 0.147 to 0.272 seconds, except that time is lacking for a complete opening of the valves before ventricular systole again causes their closure (fig. 6 *C*). The valves open slightly during the intersystolic period, the extent increasing with the As-Vs interval.

4. When the As-Vs interval is less than 0.147 seconds (fig. 6 *D*), the valves are in the process of closing due to the auricular effect when ventricular systole begins. Hence this cardiac event merely completes the closure already initiated by the auricle. There is in this case only a single closure movement, beginning before ventricular systole—a single movement, due in part to auricular contraction and in part to ventricular contraction.

VII. PHYSIOLOGICAL APPLICATION OF RESULTS

Since any conception as to the mechanisms producing valve closure must be correllated with the temporal relations of their closure to the events in the cardiac cycle, it is appropriate to discuss briefly the bearing of these results upon different theories of valve closure.

It has been shown that the mitral cusps first begin to close after auricular systole is well under way. Several possible mechanisms may account for the closure at this time. In the first place, it may be attributed to the sudden breaking of the jet of blood injected by the auricle into the ventricle, not in the manner suggested by Henderson

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and Johnson to effect the final and complete closure but rather that it tends to float the valves temporarily into position. Secondly, it is possible that the auricular contraction wave at this time reaches the muscle fibers within the valve cusps, and aids in their closure. The observation of Erlanger (4) that these muscle fibers are capable of contracting is the basis of this possibility. Thirdly, it is possible that the fall of intra-auricular pressure shown by Wiggers to occur in the middle of auricular systole (3) causes the cusps to move auricleward.

It has been demonstrated that the auricular closure of the mitral valves is incomplete and temporary—that a ventricular systole is required to effect complete closure and maintain the valve in this position until the onset of ventricular diastole. The facts that the valves close at the onset of ventricular systole irrespective of whether an auricular systole preceded, and open precisely at the beginning of ventricular diastole, lends support to the commonly accepted idea that the difference of pressure on the two sides of the valves is the chief factor in producing their movements.

It is commonly taught that the mitral cusps are approximated at the onset of ventricular systole. It has been shown in this work that some degree of approximation obtains when ventricular systole begins if the As-Vs interval falls within limits that may be considered normal (0.13–0.18 seconds). It has been further shown, however, that the extent of this approximation is directly related to the length of the As-Vs interval, so that when the As-Vs interval equals 0.272 (fig. 6 *B*) the cusps are as widely separated as at the onset of auricular systole. It is of practical importance to recognize that in cases of delayed A–V conduction the valves may undergo two distinct movements of closure, the first near the end of auricular systole, the second at the beginning of ventricular systole.

The writer wishes to express his great appreciation for the kind advice and criticism of Dr. Carl J. Wiggers.

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THE PHYSIOLOGY OF THE MAMMALIAN AURICLE

I. The Auricular Myogram and Auricular Systole

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INTRODUCTION

It is commonly believed that the auricular myogram is a graphic record of auricular systole and diastole. Such a tracing may be obtained from the mammalian auricle in several ways. Of these, the transmission of auricular movements to simple levers or tambour systems is probably the procedure most frequently employed. A small button is placed on the auricle and its movements communicated, sphygmograph fashion, to a recording lever (Ludwig and Hoffa (1)); or a point on the auricular surface is connected by a thread with a light lever or tambour system, i.e., the so-called "suspension system" is used (Gaskell (2), Englemann (3)).

As generally employed in mammalian experiments such procedures introduce two errors. In the first place, the vibration frequency of this ponderable system is so low that the records obtained are necessarily distorted by an interference of the instrument's own vibrations. This is, however, a matter of minor importance, since it is questionable whether the movements of a point or spot on the auricular surface, communicated to the most ideal lever system gives reliable evidence of the state of muscular activity which we seek to record. The auricle has no fixed point from which to contract, hence the movement of any point on its surface is modified by many factors (e.g., by lung inflation, auricular distension, changes in form, ventricular position changes, etc.) besides the contraction and relaxation of auricular fibers. It has not always been adequately recognized that changes in auricular form or position do not necessarily occur in unison with contraction and relaxation processes; that a record of one event may not be a criterion of the other.

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Recognizing these facts, a number of investigators have sought to obviate the error by recording the approximation and recession of two selected points on the auricle, independent of its position changes. To this end, myocardiographs of different design have been introduced (Roy (4), Cushny (5), Wiggers (6), Gesell (7)). These instruments have the common fault that their vibration frequency is entirely inadequate, and most of them have the additional drawback that their great mass may react upon and interfere with a normal action of the thin-walled auricle. "Die Massen der beweglichen Teile" says Frank (8) "sind viel zu gross, als dass nicht Trägheitskräfte entstehen müssten, die zu einer, die der Herzthätigkeit vollständig verändernden Rükwirkung führen müssten."

APPARATUS—THE MINIATURE MYOCARDIOGRAPH

Inasmuch as an exact myogram of auricular contraction and relaxation was demanded in a careful study of questions pertaining to the temporal relations and dynamic importance of the auricle, a miniature myocardiograph was designed which was capable of accurately following the variations in the length of auricular fibers, independent of changes in auricular form, volume or position but was incapable of reacting upon the auricle so as to make its action unnatural. A high vibration frequency and small mass are two qualifications of this apparatus. The instrument, shown in natural size in figure 1, weighs less than 2 grams and is supported by a very light spring, enabling it to follow varying degrees of auricular distension and movement without affecting the contraction curve. Connected with a Frank segment capsule, the system can have a frequency as great as 118 per second.

In the design of this myocardiograph, joints, pivots and fixed axes, which cause irregular friction and add weight, are entirely eliminated. This was made possible by using a light aluminum segment capsule, 2 cm. in diameter, covered by light but tensely stretched rubber dam. As in Frank's recording capsule, a trapezoidal aluminum plate is cemented to the rubber so that it pivots upon the chord side. This plate carries a light extension arm (1.5 cm. long) with an eyelet at its end. A second similar arm is rigidly fastened to the body of the capsule. These two arms, which may be bent so that the distance between the two eyelets varies from 3 to 25 cm. are stitched to two points on the auricular surface. The approximation of these two points causes a negative pressure in the cardiograph capsule and in the connected

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Fig. 1. Photograph of miniature myocardiograph (actual size).

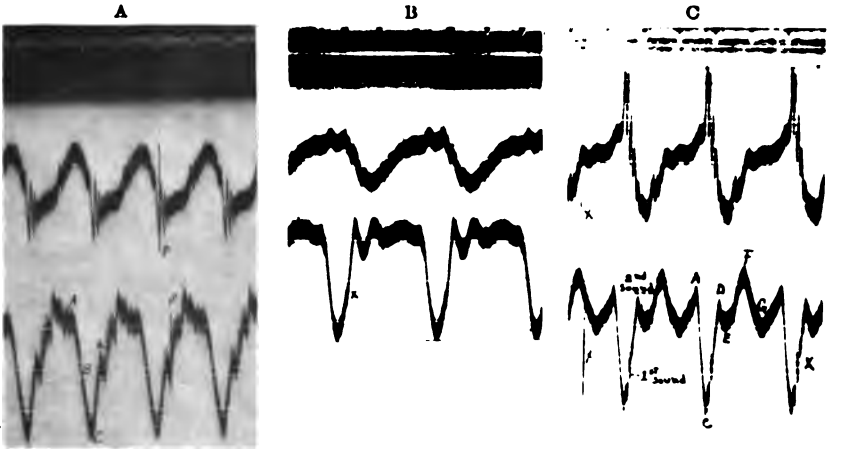


Fig. 2. *A*, *B*, *C*. Segments of myograms taken from mid-auricular region from points 5 to 7 mm. apart.

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recording capsule of Frank, hence the curves move downward during contraction and upward during relaxation. In attaching the instrument, the heart is left intact within the pericardium, a window being cut over that portion of the auricle to which the apparatus is fastened.

THE CONTOUR OF THE AURICULAR MYOGRAM

The secondary or communicated oscillations in optical myograms. It was anticipated that the myogram recorded by the miniature myocardiograph would present a very simple contour. This proved to be the case only when the auricular beat was neither preceded nor followed by a ventricular systole. Such instances are shown in waves 12 to 16 of figure 3 taken during excitation of the left vagus which had produced a complete a–v block. The curves reach their maximum contraction or trough on an average, in 0.083 second (table 1). This maximum contraction is maintained just for a moment and then the curve regains its full relaxation within approximately the same time interval. No evidence of a sustained contraction or "plateau" is present.

When the auricular beat is followed by ventricular contraction (figs. 2 to 3) the contour of the relaxation curve is altered by superimposed waves. Thus, in the three segments shown in figure 2, the ascending limb contains one or two groups of such superimposed oscillations. First, in point of time, there occurs, shortly after the onset of relaxation (X) a jog, a notch, or a series of distinct vibrations. Comparisons with the ventricular cardiogram in which the vibrations of the two sounds are present, show that this corresponds exactly with the vibrations of the first sound (p–p'). When the heart is vigorous, as after partial asphyxiation (fig. 2 C), distinct sound vibrations may be transmitted to and recorded by the instrument. It may be pointed out, parenthetically, that the ability of the recording system to reproduce such vibrations is in itself a test of its adequacy.[1]

Frequently, but not constantly, a second notch occurs toward the end of relaxation (fig. 2 C, D, E). This is apparently due to a traction from the ventricle so exerted as to cause the two points to which the myocardiograph is attached to approximate. Comparison with the

[1] It is of incidental interest to note that, when an apparatus with a low inherent frequency is used these vibrations either do not occur on the record owing to a "lever throw" or with greater damping of the apparatus the movement of the lever is arrested temporarily thus giving the curve a flattened contour suggesting a plateau. This may possibly account for the plateau curves obtained by some investigators (cf. e.g., the recent results of Ewing (9)).

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intraventricular pressure curves (to be published in a subsequent paper) show that this takes place during the early portion of the ejection period. Lastly, with the onset of ventricular diastole at *E* and again with the diastolic inflow from the auricle at the opening of the a–v valves (*F*), the auricular curves may be modified by ventricular action.

It is clear that the rebounds and position changes of the ventricular base consequent to its contraction and relaxation cause a series of slight expansions and contractions of the elastic auricle, which lies upon this base. These superimposed waves distort the correct myogram curves of auricular activity. They may vary in amplitude and number, depending partly on the vigor of ventricular systole, partly, however, on the proximity of the auricular myograph to the a–v junction. Since they are a part of the auricular movement they cannot be eliminated from any record taken by an efficient myocardiograph.

In themselves these oscillations are of no importance and would not merit the detailed discussion accorded them but for the fact that a clear understanding of their inherent cardiac and not instrumental origin makes certain the conclusion that when the heart chambers are beating in normal sequence, the contraction phase of the myogram curve alone can be recorded free from secondary vibrations of extra-auricular origin.

The mechanical as related to the fractionate contraction. It is quite certain that all the units of cardiac muscle lying between two points on the auricular surface neither begin nor cease to contract at the same time. On the contrary, to judge from the spread of the excitation wave, as established by Eyster and Meek (10) and Lewis, Meakins and White (11), the mechanical contraction recorded by a myocardiograph is the resultant of a more or less orderly series of contractions and relaxation which the fractional portions of cardiac tissue between two points undergo. We must, therefore, distinguish between the recorded *mechanical contraction* and the *fractionate contraction*, i.e., the interval during which any unit of cardiac syncitium remains in the contracted state.

Since the excitation wave, according to the American and English investigators above mentioned, spreads from the sinus node to the more distant portions of the auricle at the rate of approximately 1000 mm. per second, we should expect that the cardiac tissue underlying the more proximately placed arm of a myocardiograph starts its contraction before the tissue beneath the more distant arm. Within a

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very short interval after the onset of the contraction, the two points on the auricular surface may be expected to approximate. As more and more fractional portions of the muscle between the two arms enter into their contraction phase, we may presume that the two points approximate with a greater velocity. Lastly, when the fractionate contractions of the more proximal portions change progressively to relaxation phases, while the more distal portions remain shortened, we may expect a progressive decrease in the rate at which the two points approximate until finally, as the fractionate contractions and relaxations are equally balanced, the peak of the mechanical curve is reached.

A careful consideration of the optical myogram taken on rapid paper affords evidence that these events affect the contour of the contraction curve. Thus, as shown in waves 5 and 6 of figure 3, the mechanical contraction (A–C) can be divided by the changes in gradient, into three distinct phases. These are:

1. A proto-systolic phase lasting about 0.02 second during which the rate of contraction gradually accelerates (AA'). As interpreted by the diagram inscribed on the curve, this phase probably represents the spread of the fractionate contractions from the proximal to the distal point.

2. A meso-systolic phase ($A'B$) lasting about 0.024 second during which contraction proceeds at a uniform though maximum rate. As interpreted, this represents the interval during which all muscular tissue is contracting.

3. A tele-systolic phase (BC) lasting approximately 0.03 second during which the rate of contraction is progressively diminishing. During this stage the fractionate contractions of the more proximate portions are progressively converted into fractionate relaxations which oppose and tend to neutralize the fractionate contractions of the more distal portions of the tissue. This continues until an exact neutralization at the apex of the mechanical curve has taken place—a point which we term the end of mechanical contraction.

These three phases are followed by a phase lasting 0.03 second or less, during which the curve turns upward with a very gradual gradient owing to the fact that the fractional relaxations are beginning to predominate over the fractional contractions (CD).

If these interpretations prove correct then the apex of the mechanical contraction does not indicate the moment when the fractionate contractions have all ceased. At B some of the fractions of cardiac tissue have started to relax and to a distance beyond C other fractions con-

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tinue to contract. Since the initiation of contraction at the proximal arm of the myocardiograph is indicated by the onset of the mechanical curve at *A* and the first evidence of relaxation at this point is indicated by a change of contour at *B*, it will probably be fairly accurate and allowable to estimate *the duration of the fractionate contraction* of the more proximal tissue from the period *AB*.

THE RELATION OF THE MYOGRAM TO AURICULAR SYSTOLE

The term "systole" (Greek, *συστολή*, a drawing together or shortening) is best defined by current usage as the period of mechanical shortening of the entire auricular musculature. So used, the term is evidently not synonymous with, but much longer than the fractionate contraction interval. The question remains: Does the myogram recorded from two points on an anterior auricular surface give the full systolic interval? It is obvious that the duration of the mechanical contraction recorded by the myogram will depend directly on the distance between the two points selected for its attachment. In other words, the shorter the distance between the two points selected, the more nearly the mechanical contraction recorded will approximate the fractionate contraction; the farther they are apart, the more nearly will the contraction correspond to the total auricular systole. It is obvious that when it is desirable to study the influence of nerves or chemicals on the functions of irritability and contractility, the former procedure is preferable; whereas, when it is desirable to make time comparisons with the systole of the auricle, the greatest possible distance should theoretically be chosen between the two approximating points. Following the evidence given by the spread of the excitation wave as established by Eyster and Meek (10) and Lewis, Meakins and White (11) the two points to be selected for obtaining the full auricular systole would be the sinus node and the tip of the auricular appendix. Unfortunately, however, it is not feasible in practice to record a reliable myogram from points lying in two different planes. The largest area that is feasible experimentally, consists of a stretch between the right border of the auricle and the auricular tip (maximum, 28 mm., in a large dog). Even when this is chosen, the curve is somewhat deformed by changes in the form of the auricular surface.

It is necessary, therefore, to determine (1) whether there exists an essential time difference between the mechanical contraction recorded from two near and two distant points; and (2) how much the first auricular activity precedes that recorded by the myogram.

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In relation to the first question, experiments show that the end of mechanical contraction is reached distinctly sooner in curves recorded from points 3–4 mm. apart than in those recorded from those separated 25 mm. or more. On the other hand, no time difference, as regards the end of contraction, could be found between two myograms taken respectively from points separated 25 mm. and 5–7 mm. The only explanation suggesting itself for this is that in an area 5 mm. wide there is as good a balancing of contracting and relaxing fractions as over wider areas. Hence, nothing is gained by using points separated more than 10 mm., provided they are not selected on or toward the auricular appendage, for if this is done the onset is delayed.

The second question, as to how much the first mechanical activity of the auricle precedes the myogram curve, may be most satisfactorily answered by comparing the auricular myogram with the intraauricular pressure curve. This was recorded by the type of optical manometer previously described by the writer (12). The cannula of this instrument was inserted into the azygos vein and pushed into the auricle via the superior vena cava.

A study of such records (fig. 3, curve 4) shows that the intraauricular pressure begins to rise 0.014 to 0.033 second (average 0.022) before the myogram curve begins to descend (cf. table I). The conclusion is evident that *the myogram does not record the complete interval of auricular* systole. In the comparison of time relations with auricular systole, it is therefore necessary to record a simultaneous intraauricular pressure curve or somewhat less accurately add to this period of mechanical contraction of the myogram the average interval 0.022 second (see table 1).

Dynamic period of auricular systole. A further comparison of the myogram and the intraauricular pressure curve (fig. 3) shows that the pressure within the auricle does not continue to rise during the entire interval of auricular shortening but reaches its maximum or summit when the contraction curve has been only partly completed. Close inspection (cf. wave 4 of figure 3) shows that this maximum occurs approximately when the change in the gradient at B appears, i.e., where relaxation of some auricular muscle has already begun, although the resultant mechanical effect is still one of shortening. The duration of this rise averages 0.053 second (table I). It is apparent that only the early half of auricular systole is effective in producing a rise of intraauricular and presumably of intraventricular pressure. This may, therefore, be designated as the *dynamic period* of auricular systole.

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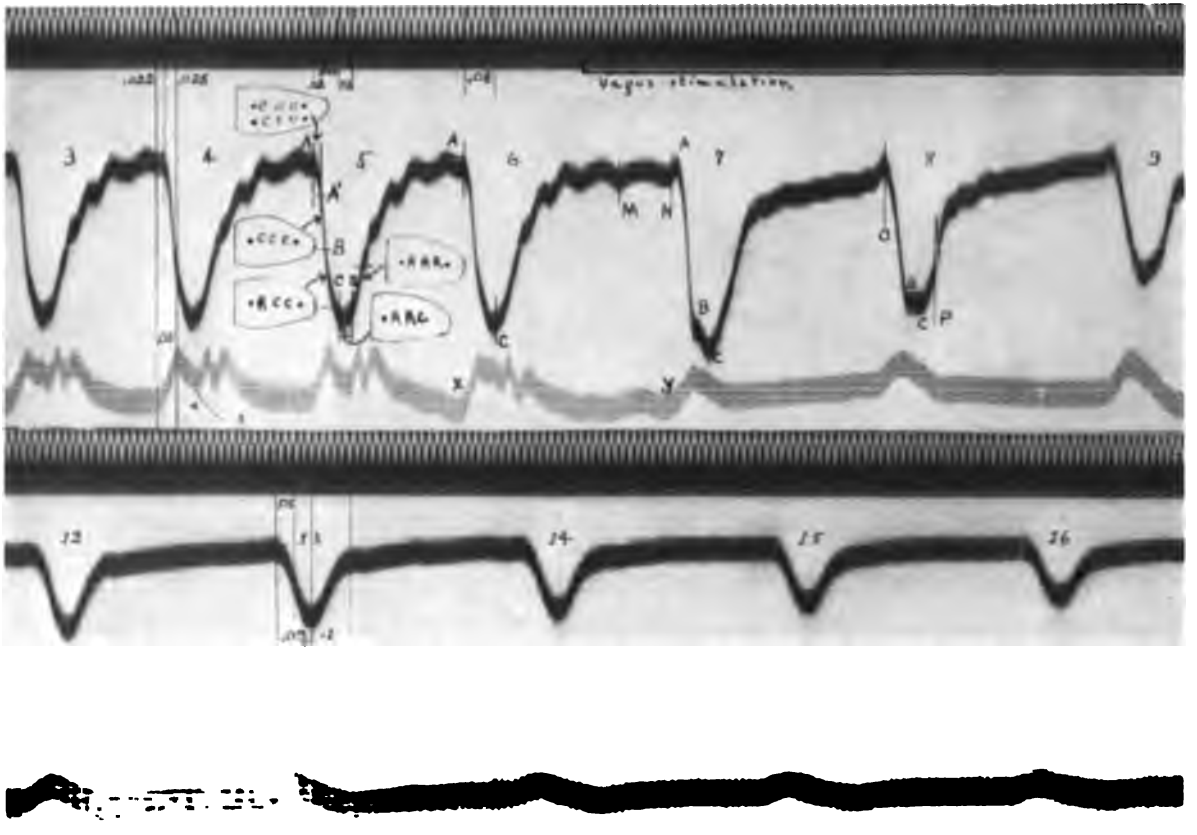


Fig. 3. Myogram of heart and synchronous intra-auricular pressure curve on rapid drum taken from points 12 mm. distant during normal action and during stimulation of left vagus. Waves are numbered from 3 to 16, in order to facilitate reference in text. Turning fork period equals 0.02 second. In the interpretative diagrams appended to wave 5, the two dots represent the points of attachment of the myocardiograph arms. *C* expresses a state of local contraction; *R*, relaxation; and *O* of rest.

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Inasmuch as the dynamics of the heart beat are concerned with the maximum tension developed by auricular contraction rather than by its duration, this dynamic period and not the interval of auricular systole should be used when questions of dynamics are concerned.

When a ventricular contraction follows that of the auricle after a short interval, the fall of the auricular curve (extending into auricular diastole as sketched in at α β in wave 4 of figure 3 when the ventricle is not beating) is terminated by a sharp rise and fall due to ventricular activity. Its cause need not be considered here. Here, the entire rise and fall of the normal auricular wave within the auricle is completed during systole of the auricle. This confirms the results obtained recently by Ewing (18).

THE TIME RELATIONS OF AURICULAR EVENTS

The time relations of auricular events, as gathered from different experiments carried out under similar conditions are presented in table A. They show that the mechanical contraction recorded from the mid-auricular region or entire anterior surface of the auricle varies

TABLE A

EXPERIMENT	(1) FRACTIONATE CONTRACTION	(2) TOTAL MECHANICAL CONTRACTION	(3) INTERVAL BETWEEN RISE OF AURICULAR WAVE AND MYOGRAM	(4) TOTAL SYSTOLE (2+3)	(5) DYNAMIC SYSTOLIC INTERVAL
C75, VIII		0.070			
C76, IX	0.050	0.094			
C77, VI	0.062	0.093	0.033	0.126	0.038
C78, III	0.032	0.066	0.014	0.080	0.042
C77, IV		0.096	0.030	0.126	0.062
C80, XV	0.037	0.077	0	0.077	0.055
C81, VI	0.059	0.088			
C83, III		0.051			
C84, I		0.120	0.020	0.140	0.075
C88, I		0.078			0.050
C89, I		0.066			0.048
C90, III					0.060
C92	0.051	0.095			
C93	0.055	0.108	0.016	0.124	
C94		0.065			
C95	0.046	0.081			
C96	0.044	0.080	0.022	0.102	0.050
Average.....	0.0469	0.083	0.022	0.01107	0.0533

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from 0.05 to 0.12 second, an average of 0.083 second. The interval from the onset of mechanical contraction to the second change in gradient, probably indicating the duration of fractionate contraction process, varied from 0.032 to 0.062 second, averaging 0.0469 second. The average of nine experiments shows that the intraauricular pressure curve precedes the myogram curve about 0.022 second, the shortest interval being 0.014, the longest 0.033 second. Adding these figures (or in cases where a simultaneous intraauricular curve was not taken, the average figure 0.022 second) it is found that the duration of the entire auricular systole averages 0.11 second, the widest variations being 0.077–0.140 second. The auricular wave of the intraauricular pressure curve reaches its maximum on an average within 0.053 second, the widest variation being from 0.038 to 0.075 second. Evidently the dynamic period of systole extends only over little more than the early half of auricular systole.

SUMMARY

1. The excitation wave, to judge from the appearance of negativity over different points of the auricle, spreads from the sinus node to more distant portions of auricular muscle. A short interval after each unit of cardiac muscle is so excited, it begins to contract. This local contraction, termed the *fractionate contraction* has an average duration of 0.469 seconds.

2. When the approximation of two points on the auricle is recorded by a myocardiograph, the myogram so obtained gives evidence in the changing rate of shortening that the onset of contraction develops at one point before it does at the other and spreads to the second attachment, within a period of approximately 0.02 second; after which the entire musculature between the points continues to shorten for 0.024 second. The remainder of the curve represents the resultant between the fractions continuing to contract and those that have already started to relax. This series of events represented by the mechanical contraction recorded by the myogram takes about 0.083 second. After a balance has been reached the two points begin to separate, at first slowly but later when all fibers have started to relax, more rapidly.

3. The interval of *mechanical contraction*, as established by the myogram, does not give the complete interval of auricular systole. This is evidenced by the fact that the intraauricular pressure curves rises

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approximately, 0.022 second earlier. This interval added to the mechanical contraction, makes the average period of *systole* equal to 0.1107 second.

4. Auricular systole continues to develop tension, as indicated by the rise of the intraauricular pressure curve, only as long as all muscular units continue to contract. As soon as evidence appears in the myogram that the curve is a resultant of fractionate contraction and relaxation processes, the intraauricular pressure curve falls. The period during which tension is developed lasts about 0.053 second and may be designated as the *dynamic period of auricular systole.*

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NOTE ON PROTECTION OF STRING GALVANOMETER CIRCUITS AGAINST EXTERNAL ELECTRICAL DISTURBANCES

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The use of such an instrument as the string galvanometer which is capable of detecting very feeble electrical currents, even those of brief duration, is beset with special difficulties. Not the least of these is the difficulty of preventing disturbance of the instrument by adjacent electrical apparatus, motors, lighting circuits and the like. Not only is the galvanometer apt to be affected by the proximity of other electrical apparatus, but the electrostatic charges on rubber insulation, the clothing of the operator and other objects may become a source of trouble in a manner presently to be indicated. The disturbances that may arise from faulty insulation are perhaps more generally understood and will not be considered in this note. Granting that the insulation of the galvanometer circuit is adequate, external electric currents and charges can affect the instrument only by induction. We shall first consider

ELECTROMAGNETIC INDUCTION

Whenever a conductor which forms part of a closed circuit moves in a magnetic field in such a way as to vary the number of lines of force enclosed by the circuit, a current will be produced in the circuit. Also if a wire forming part of a closed circuit is so placed in a magnetic field as to enclose some of the lines of force, a variation of the strength of the field will alter the number of lines of force enclosed by the wire and a current will thus be set up in the wire though the wire itself is stationary. A few concrete examples may help to make clear the bearing of the above statements upon protection of galvanometer circuits. The wires which lead to and from the string terminals of a string galvanometer always pass through the relatively strong stray field of the instrument itself. If these wires quiver ever so slightly,

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say with vibrations of the building or of motors used in connection with the apparatus, they will cut lines of force and set up currents through the string which become evident as a to and fro movement of its shadow having the same period as that of the vibrating wires. If any part of the string circuit runs near a wire transmitting alternating current for power or lighting, the variations in the magnetic field surrounding the power wire induce currents which cause the string to move to and fro with a frequency identical with that of the alternations of the power current. If a wire of the string circuit runs near a wire which supplies continuous current to the arc lamp usually used with string galvanometers, the string will move with every fluctuation of current in the lamp.

The easiest way to prevent trouble from movement of wires in the stray field of the instrument is to use very stiff wires and to fasten them so that the liability of movement is minimized. In other parts of the circuit the effect of varying fields, or of movement of the wires in steady fields, can be made very small by twisting together the (insulated) going and returning wires of the string circuit. Suppose the pole of a permanent magnet to be so moved that the number of lines of force in the region of an adjacent pair of wires is varied. If the wires are separated an appreciable distance they will enclose lines and the number of lines enclosed will vary as the magnet moves. According to the general statement made at the beginning of this section, a current in the wires will result. If, however, the wires are run close together so that very few lines can pass between them, then unless the field is very strong, the current produced by its variation, or by movement of the wire through it, will be feeble. If in addition the wires are twisted together, the spaces left between them will be a series of figures of "8" and a moment's reflection will show that the effect of a variation of the number of lines of force in one of the loops of the eight will be exactly compensated if the same variation occurs simultaneously in an adjacent loop. If a circuit is enclosed in an iron tube, magnetic lines external to it tend to flow in the wall of the tube on account of the great permeability of iron as compared with air. Only a very small number of lines will reach a circuit so enclosed. If the enclosed wires be also twisted, the protection will be still better. The thicker the iron wall, the more nearly complete will be the protection afforded. The protection is in no case absolute, but by sufficiently increasing the thickness of the armor it can be made as great as necessary. It is desirable that all power and lighting wires in the immediate neigh-

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borhood of a string galvanometer equipment shall be enclosed in iron pipe and if such wires are also twisted together the magnetic effect of variable currents in them upon outside circuits will be further diminished. Wherever possible the outgoing and return wires of the string circuit should be twisted together and if necessary this circuit may be enclosed in iron also.

ELECTROSTATIC INDUCTION

If an insulated charged body is brought into the neighborhood of an insulated neutral body, an alteration of the distribution of electricity upon the latter occurs as illustrated in figure 1. While this change of distribution is taking place a momentary current will flow in the neutral body. If a second neutral body is brought into the neighborhood of the other two, there will be another change in the distribution of electricity on the first neutral body with another transient current, and so on. Consider the neutral body to represent the insulated wires, resistance boxes, switches, commutator, etc., connected to the string. Let the charged body be the hard rubber handle of one of the resistance boxes, charged by the friction of the operator's hand. Each increase or diminution of the charge will cause change in the distribution of electricity in the string circuit and the passage of a momentary current through the string. Once some part of the apparatus has acquired a charge, the mere movement of the operator's hand near the charged part is sufficient to disturb the electrical distribution and cause the passage of current through the string. Attention was directed to disturbances of this character by Einthoven in one of his earliest communications regarding the string galvanometer (1). Movement of any conductor near a charged body close to the string circuit will cause these disturbances. The conductor may be part of a piece of machinery, say a wheel connected with the apparatus for recording the movements of the string. In this case the disturbance will be rhythmic. It is unnecessary to multiply examples, as anyone who has worked much with these instruments will recall similar occurrences in his own experience. Not only do these disturbances occasion annoyance and sometimes lead to confusion

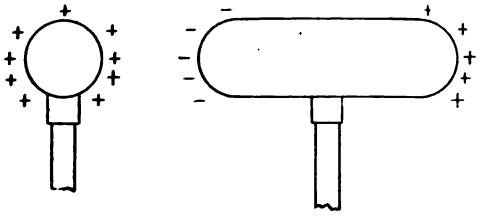


Fig. 1

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in the interpretation of experimental results, but they are often of quite sufficient intensity to cause the loss of a string.

Electrostatic disturbances can be entirely eliminated by the method of "electrostatic screening." If an insulated conductor be completely enclosed in a hollow conductor which is at zero potential, the conductor within is entirely unaffected by variable or moving charges external to the hollow conductor.[1]

The hollow conductor at zero potential is called an electrostatic screen. For practical purposes it is not necessary that the hollow conductor be everywhere continuous. A wire mesh enclosing the apparatus to be protected is nearly as effective as a solid sheet of metal. The condition of zero potential is approximately satisfied by connecting the screen to the earth. In the practical application of the method to a galvanometer circuit it is customary to ensheath the wires of the string circuit in lead. Lead covered insulated wires are readily obtainable from dealers in electrical supplies in the larger cities. Such wire has the further advantage that it is so stiff and inelastic as to minimize the difficulties mentioned in the first section as arising from vibration of wires in an adjacent magnetic field. Rheostats and switches may be enclosed in boxes of sheet tin and the rheostats which have rotary contacts may be covered with wire gauze provided with perforations for the handles. The latter if of hard rubber may be covered with tinfoil. Plug rheostats are less easy to protect and inasmuch as boxes with rotary contacts can now be obtained of excellent quality at small expense, it is better to select them for this service. All the tin boxes, wire gauze, lead coverings of wires and the like should be earthed, preferably all to the same point. Usually connection to a water main gives a sufficient earth. Care should be taken that the connection is substantial as it is likely to become loose or corroded in time if made by merely twisting a wire about the pipe. A clamp is better.

NEW APPARATUS FOR COMPENSATION AND STANDARDIZATION.

The writer has had frequent opportunities to observe the inconvenience and inadequacy of apparatus supplied for compensation and standardization by the makers of string galvanometers. Most of these instruments are needlessly expensive and in many of them effective screening would be quite impossible.

[1] For the mathematical proof see J. J. Thomson, Elements of the mathematical theory of electricity and magnetism, 3d ed., 1904, p. 51–52.

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Figures 2 and 3 are reproduced from photographs of an apparatus which has been developed by the Leeds and Northrup Company of Philadelphia to meet the writer's specifications for an adequate outfit of resistance boxes to be used in connection with a string galvanometer. It has been arranged primarily to meet the requirements of those who use the galvanometer for the clinical study of the heart, but will be found suitable for nearly every necessity which attends the use of the galvanometer in the physiological laboratory. The general arrangement is similar to that of the apparatus which has been in use for several years in the physiological laboratory of Columbia University and does not differ greatly from the scheme published by Einthoven (2). The noteworthy feature of the apparatus is that all the rheostats, switches and commutator are controlled by rotary handles and that coils and contacts are completely enclosed in a copper-lined box. All the handles are of metal which is in metallic connection with the copper lining and by connecting to earth the terminal marked "Earth," the entire apparatus is effectively screened. The binding posts for making connection to other apparatus can not be covered without difficulty, but they are placed at the back of the box, away from the region where the operator is likely to move his hands. One of these boxes was connected to a string galvanometer and the earth terminal purposely left disconnected. A light rubbing of the hard rubber top of the box with the finger was sufficient to cause the string to deflect so far that it came in contact with the rear wall of the space in which it moves and remained fast there until cautiously dislodged. After connecting the earth terminal to earth the top of the box was polished with a piece of silk without causing the slightest movement of the string.

The principle of the apparatus is illustrated in figure 4. An ordinary dry cell is connected in series with a resistance, R_1 which consists of a fixed resistance of 10,000 ohms and sufficient variable resistance to permit of adjusting the current which the cell will drive through, to the value 0.0001 ampere. The sensitive galvanometer, G, enables one to know when this amount of current is flowing. The resistance R_2 forms a derived circuit and when the current in R_1 has the value 0.0001 ampere, there will be a difference of potential of 0.001 volt across the terminals of R_2 for every 10 ohms introduced into R_2. This last statement requires the qualification that it is very nearly true unless the amount of resistance introduced into R_2 is an appreciable fraction of the resistance of R_1. In that case the current in R_1 will depart sensibly from the value 0.0001 ampere, but it can always be brought back to

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Fig. 2



Fig. 3

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that value by readjustment of the variable part of R_1 so that the standardization can be kept as accurate as the accuracy of the small galvanometer, G. This has an accuracy of 1 per cent. The greatest accuracy attainable in work with the string galvanometer is not more than 2 per cent, so that the galvanometer error is within the limit.

Referring to figure 3, the knob at the upper left hand corner marked "AB" is a commutator for the standard current. Below it is the galvanometer, "G." The two knobs marked, "Regulator for standard voltage," control the variable part of R_1. The four knobs marked "Compensating and standardizing resistance" control the resistance R_2. The terminals marked "$R.A.$" $L.A.$" etc., are for connection to the

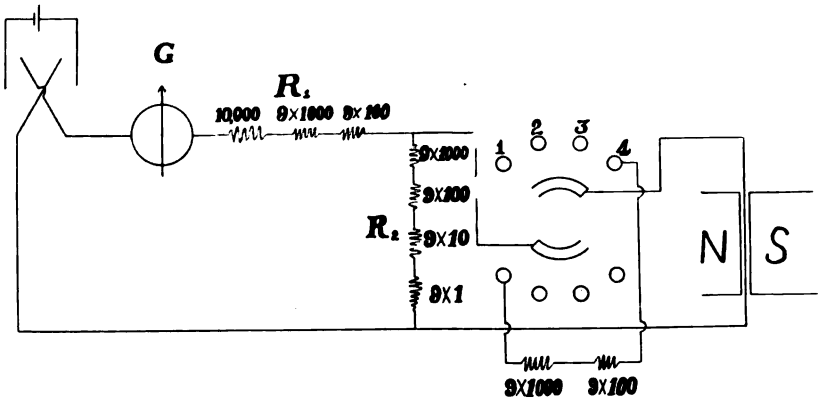


Fig. 4

extremities of the patient for clinical work and may naturally be connected in any convenient manner for laboratory purposes. In the lower right corner is a knob with a pointer and a series of figures, 1, 2, 3, 4. When the pointer is at 1, the galvanometer will be connected to the terminals $R.\ A.$ and $L.\ A.$, when at 2, to $R.\ A.$ and $L.\ L.$ following the usual manner of numbering the leads adopted by Einthoven for study of the heart. When the pointer is at 4, the galvanometer is connected to the resistance marked "Comparison resistance" which may be used to measure by substitution the resistance of a patient or physiological preparation. At the upper right corner is a knob which controls a resistance of 100,000 ohms and 10,000 ohms which may be put in series with the string during compensation to diminish the deflection.[2] The position for this knob when the instrument is out of use is at the point

[2] Not indicated in figure 4.

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marked "Inf." This disconnects the galvanometer entirely and prevents the large throw of the string when the field magnet circuit is broken which is so apt to destroy strings in apparatus where a shunt resistance is used to cut down the sensitiveness during compensation.

SUMMARY

Methods of protecting string galvanometer circuits against electromagnetic and electrostatic disturbances are discussed and a brief description appended of an arrangement of resistances for use with string galvanometers in which effective electrostatic screening has been provided in the construction.

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ON THE ACTION OF CERTAIN SUBSTANCES ON OXYGEN CONSUMPTION

I. The Action of Potassium Cyanide

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This paper is the first of a series intending to deal with the effect of a number of substances, especially anaesthetics, on the rate of oxygen consumption. My interest in this problem was aroused through the use of such substances as experimental agents for altering the rate of metabolic processes, for demonstrating the existence of metabolic gradients in organisms, and in the control of morphogenesis.[1] The present paper deals entirely with the action of potassium cyanide, a substance which has proved of peculiar value for the above purposes. It is hoped in the future to test similarly the action of alcohols, ether, chloroform, urethanes, acids, alkalies, and some salts.

A number of experiments have already been performed to determine the effect of cyanogen compounds on living matter. The classical experiments were those of Geppert (1), who in 1889 showed that the expired air of mammals under hydrocyanic acid poisoning contains more oxygen than normally, and, further, that the venous blood has a higher oxygen content than under normal conditions. From these results, Geppert concluded that cyanogen compounds act by reducing the capacity of the cells for consuming oxygen. Since these experiments it has been widely accepted that the cyanides produce their effect on protoplasm by directly depressing the oxidation processes. Geppert's work is, however, open to two criticisms. In the first place, it was long ago suggested that the cyanides may act by uniting with the oxygen-carrying compound of the blood, just as carbon monoxide does. Zeynek (2), however, has demonstrated that cyanides will not unite with haemoglobin at all, and with oxyhaemoglobin only after

[1] For a complete discussion of these matters, consult Child (5) and (6), where further references will be found.

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heating several hours at body temperature. This criticism is, therefore, invalid. On the other hand, the work of Grove and Loevenhart (3) has shown that the action of cyanides in the case of mammals is primarily upon the respiratory center, and the reduced oxygen consumption observed by Geppert may be due in part to the general depression of the respiratory mechanism. Mammals are therefore unfavorable objects for this kind of experiment.

Nevertheless, subsequent work has entirely justified Geppert's conclusion. The best experiments that I know of are those of Schroeder (4) on the fungus Aspergillus niger; his data show a very marked decrease in both oxygen consumption and carbon dioxide output in the presence of rather strong concentrations of potassium cyanide. A number of other experiments have also been performed with cells, mainly egg cells, and with parts of animals;[2] but most of these have assumed rather than attempted to prove that the cyanides decrease oxygen consumption, although the results have always justified the assumption.

I thought it worth while to test directly the effect of potassium cyanide on some simple animal, since the only other direct measurements on animals have been performed on mammals; and to use a wider range of concentrations in order to determine whether or not very dilute solutions have a stimulating effect on oxygen consumption. The stimulating effect of potassium cyanide in very dilute concentration has already been noted by Lyon (7) who found an acceleration in the rate of development of the sea-urchin, and by Gasser and Loevenhart (8) who observed that the primary effect of cyanide on the medullary centers is a stimulating one.

The work was done at the Puget Sound Marine Station, Friday Harbor, Washington, where the University of Chicago kindly provided me with a research room.

MATERIAL AND METHODS

For these experiments it was thought advisable to select an animal which, first, has no muscular system, since variations in the degree of muscular activity are a source of error, and, secondly, no circulatory system, in order to avoid any possibility of an action of the cyanide on the oxygen-carrying compound of the blood. The only animals of sufficient size which fulfil these requirements are the sponges, al-

[2] References to this literature will be found in Child (5), p. 66.

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though even they are capable of slight muscular activity. I found in the Friday Harbor region a heterocoelous calcareous sponge of the genus Suberites which proved well adapted for my purposes. These sponges were obtained by dredging at a depth of 60 to 90 meters, and were kept in a float car moored near the laboratory. The largest individual used measured 75 x 30 x 45 mm.; the others were somewhat smaller than this.

The sponge Suberites, unlike most sponges, is not attached to the bottom, but grows upon a shell inhabited by a hermit crab. It soon dissolves away the shell leaving within itself a spiral cavity which continues to be occupied by the crab. This sponge is therefore remarkably free from dirt and debris, and can be dredged without the usual injury attendant on separating a sponge from its substratum. For both of these reasons, Suberites seemed the most suitable of the available sponge material. The hermit crab must, of course, be removed.

The experimental procedure was very simple. Two wide-mouthed bottles, of about 1500 cc. capacity, were employed, one for a control, the other for the experiment. Each bottle was provided with a tightly fitting rubber stopper pierted by three glass tubes. Two of these tubes extended to the bottom of the bottle, one for the entrance, the other for the exit of water; the third tube extended only to the level of the stopper, and was used to admit air during the siphoning of the samples used for the oxygen determination. All connections were made as tight as possible.

The experiments were carried out on a raft fastened to the laboratory dock. Each experiment consisted of six half-hour determinations, three of normal respiration, and three of respiration in the presence of various concentrations of potassium cyanide. Fresh sea-water dipped up near the raft, and fresh cyanide, weighed out immediately before use, were employed for each of the half-hour determinations. The normal or cyanide-containing sea-water was siphoned simultaneously into both control and experimental bottles, the latter containing, of course, a sponge. The bottles were then suspended in the water at about a foot and a half below the surface (in order to secure a constant temperature). At the end of the half-hour period, they were drawn up, and a sample siphoned off from each into a small bottle, care being taken to secure a thorough sample by letting the bottle overflow for some time. These samples were then analyzed for oxygen content. A uniform order of procedure was adopted and maintained throughout all the experiments.

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The oxygen content was determined by the Winkler method as given by Birge and Juday (9). Tests showed that the same quantities of the reagents may be used with salt as with fresh water. The thiosulphate was standardized with potassium permanganate instead of potassium bichromate because of the greater ease of determination of the end point.[3]

It is believed that all serious sources of error have been avoided. The principal precautions which were taken may be listed as follows:

1. The use of a control bottle of sea-water kept under the same conditions as the experimental bottle eliminates the possibility of error due to oxygen consumption by the numerous micro-organisms present in the water.

2. A practically constant temperature was maintained by immersing the bottles in the water. The temperature of the sea-water at the Friday Harbor station is very constant; during the four weeks occupied by the experiments, it varied between 11.2 and 13.7°C. The greatest variation recorded during the four hours required for each experiment was 0.9°, and a variation of this extent occurred only once. As these temperatures were necessarily read at the surface, it is certain that at the depth where the bottles were suspended the temperature varied even less than this.

3. The sea-water was shaken up vigorously before being used in order to secure a more uniform oxygen content. The oxygen content of the water near the laboratory varies greatly but for some unknown reason never attains the value which it should have at its temperature and salt content (according to the tables in Murray and Hjort (10)). The oxygen content of the shaken sea-water, in something over a hundred determinations, lay between 4.8 and 5.2 cc. per liter, being generally about 5 cc.

4. As regards the Winkler method, the chief criticism against it is the possibility of absorption of iodine by organic or inorganic substances (as H_2S) which may be present in the water; this would lower the oxygen value obtained. This source of error need not be con-

[3] To 100 cc. of distilled water add several grams of potassium iodide. (amount depends on the concentration of the standard solutions used). Acidify with a few drops of concentrated sulphuric acid and add a known, carefully measured amount of the standard permanganate. Titrate the iodine set free with the thiosulphate solution which is to be standardized using the usual starch indicator. This method is due to Prof. J. Stieglitz of the Kent Chemical Laboratory, University of Chicago. This laboratory also kindly furnished me with the standard permanganate used.

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sidered here since it enters equally into the determinations in both normal and cyanide-containing sea-water, and does not affect the relative value of the results. Nevertheless, I have tested the iodine absorption of the sea-water directly by adding a small amount of standard iodine and titrating back with standard thiosulphate. In spite of the fact that considerable organic refuse is discharged into the water from the near-by salmon cannery, the iodine absorption of the sea-water is so extremely small as to be entirely negligible; nor do the sponges add any appreciable amount of iodine absorbing material to the sea-water.

5. In siphoning off the samples, it is necessary to admit atmospheric air. This may introduce an error as the water in the experimental bottle might take up oxygen during the siphoning, since its oxygen content has been reduced by the sponge. In order to minimize this source of error, the water was withdrawn from the very bottom of the bottles, the siphoning was conducted as rapidly as possible without any agitation of the bottles, and the siphoning was stopped when about half of the water in the bottles had been withdrawn. It is therefore highly improbable that any oxygen could have penetrated to the water which was siphoned off for analysis. Furthermore, this would be against rather than in favor of the purpose of the experiments as it would tend to reduce the difference between normal oxygen consumption and oxygen consumption in the presence of cyanide. The error could be avoided by having two experimental bottles each containing a sponge, and running the water from one into the other in siphoning. I tried this once or twice but the manipulation proved so cumbersome and the results so little different from the other method that I gave it up.

As a matter of fact, the chief difficulty encountered in the experiments was the behavior of the animals themselves, as will appear in the next section.

NORMAL OXYGEN CONSUMPTION OF THE SPONGES

It soon became evident that the normal oxygen consumption of any one individual sponge may vary greatly in successive half-hour determinations. This was an unexpected and undesirable state of affairs, since it would make the results with cyanide inconclusive. I discovered after a while that the cause of the variation in oxygen consumption lies in the condition of the oscula. Each sponge possesses on its upper side one to three large oscula, which are capable of contraction. When the sponge is stimulated, the oscula close slowly,

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and remain closed for some time, after which they gradually relax again. In my experiments, the handling necessary in placing the sponge in the experimental bottle almost invariably brought about a closing of the oscula. The oscula then gradually opened until by the third half-hour, they were widely expanded, and remained so throughout the three following half-hour determinations.

When the oscula are closed, the oxygen consumption is diminished. The following data show this very clearly.

Experiment 14

	OXYGEN CONSUMED IN NORMAL SEA-WATER	CONDITION OF THE OSCULA
	cc.	
First half hour	0.54	Closed
Second half hour	0.85	Partly closed
Third half hour	1.22	Open

Experiment 15

First half hour	1.85	Open at beginning, closed at end
Second half hour	0.61	Closed
Third half hour	2.02	Open

Experiment 17

First half hour	1.64	Open
Second half hour	1.24	Partly closed
Third half hour	1.82	Open

Experiment 19

First half hour	0.52	Closed
Second half hour	2.09	Open
Third half hour	1.63	Open

Experiment 20

First half hour	0.29	Partly closed
Second half hour	1.19	Open
Third half hour	1.87	Open

Two possibilities suggest themselves to me as to the cause of the diminished oxygen consumption when the oscula are closed—either the activity of the cells of the sponge is diminished, or the decrease in oxygen content does not became apparent in the water in the bottle

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because of the cessation of the water current through the sponge. In other words, the sponge may be using up the oxpgen in the water which fills its spaces, but since the oscula are closed, this water does not get to the outside. If this were the entire explanation, the apparent oxygen consumption when the oscula first open ought to be greater than it is later when the oscula have been expanded for some time. As this does not appear to be always the case, it is probable that both factors enter into the result.

The contractile oscula may be used for determining the irritability of the sponge by the familiar method of measuring the reaction time which elapses between the application of a stimulus, and the closure of the oscula. It was my intention to test in this way the effect of various concentrations of potassium cyanide on the irritability of the animals but owing to lack of time I was able to perform only a few rather hasty experiments. These indicated that the irritability is little if at all affected by the dilute concentrations but is considerably decreased in the strong concentrations of cyanide.

EFFECT OF POTASSIUM CYANIDE ON OXYGEN CONSUMPTION

The above observations show that it requires about one hour after the sponge is placed in the bottle for the oscula to resume their normal state of expansion. Although three successive determinations of normal oxygen consumption were made in each experiment, the first two have been discarded, and the figure for the third half-hour has been taken as representing the normal rate of oxygen consumption. It is this figure which appears in the tables below. Following this figure are three successive half-hour determinations of oxygen consumption in the presence of a certain concentration of potassium cyanide. As the importance of the condition of the oscula was not realized at first, no note of their state of expansion was made in the earlier experiments. In experiments 2 to 12, inclusive, it is not therefore certainly known that the oscula were open during the determinations but there can be but little doubt of this; in experiments 13 to 21, inclusive, I do know that the oscula were widely expanded throughout the four half-hours for which the figures are given. The data are arranged in the order of the concentration of cyanide used. The stated concentrations of the cyanide solutions are approximate only. The experiments starred were performed on the same individual sponge.

It should be added that complete recovery from the effect of the cyanide occurred in every case.

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Experiment 5

	OXYGEN CONSUMED cc.
Half-hour in normal sea-water	0.72
First half-hour in 0.000005 mol. KCN	0.86
Second half-hour in 0.000005 mol. KCN	1.00
Third half-hour in 0.000005 mol. KCN	0.93

Experiment 3

Half-hour in normal sea-water	0.39
First half-hour in 0.00001 mol. KCN	0.41
Scond half-hour in 0.00001 mol. KCN	0.23
Third half-hour in 0.00001 mol. KCN	0.16

Experiment 6

Half-hour in normal sea-water	0.88
First half-hour in 0.00001 mol. KCN	0.88
Second half-hour in 0.00001 mol. KCN	0.52
Third half-hour in 0.00001 mol. KCN	0.65

Experiment 11

Half-hour in normal sea-water	1.14
First half-hour in 0.00001 mol. KCN	1.14
Second half-hour in 0.00001 mol. KCN	1.32
Third half-hour in 0.00001 mol. KCN	1.16

Experiment 7

Half-hour in normal sea-water	0.39
First half-hour in 0.00002 mol. KCN	0.14
Second half-hour in 0.00002 mol. KCN	0.12
Third half-hour in 0.00002 mol. KCN	0.13

Experiment 8

Half-hour in normal sea-water	0.74
First half-hour in 0.00002 mol. KCN	0.80
Second half-hour in 0.00002 mol. KCN	0.66
Third half-hour in 0.00002 mol. KCN	0.12

Experiment 12

Half-hour in normal sea-water	1.26
First half-hour in 0.00002 KCN	1.30
Second half-hour in 0.00002 KCN	0.93
Third half-hour in 0.00002 KCN	0.87

*Experiment 13**

Half-hour in normal sea-water	1.55
First half-hour in 0.00002 KCN	1.72
Second half-hour in 0.00002 KCN	1.08
Third half-hour in 0.00002 KCN	0.99

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Experiment 9

	OXYGEN CONSUMED cc.
Half-hour in normal sea-water	0.93
First half-hour in 0.00004 mol. KCN	1.06
Second half-hour in 0.00004 mol. KCN	0.40
Third half-hour in 0.00004 mol. KCN	0.39

Experiment 14

Half-hour in normal sea-water	1.22
First half-hour in 0.00004 mol. KCN	1.09
Second half-hour in 0.00004 mol. KCN	0.88
Third half-hour in 0.00004 mol. KCN	0.69

*Experiment 15**

Half-hour in normal sea-water	2.02
First half-hour in 0.00004 mol. KCN	1.09
Second half-hour in 0.00004 mol. KCN	0.83
Third half-hour in 0.00004 mol. KCN	0.62

Experiment 2

Half-hour in normal sea-water	0.64
First half-hour in 0.0001 mol. KCN	0.14
Second half-hour in 0.0001 mol. KCN	0.18
Third half-hour in 0.0001 mol. KCN	0.14

Experiment 10

Half-hour in normal sea-water	0.98
First half-hour in 0.0001 mol. KCN	0.50
Second half-hour in 0.0001 mol. KCN	0.13
Third half-hour in 0.0001 mol. KCN	0.20

*Experiment 17**

Half-hour in normal sea-water	1.82
First half-hour in 0.0001 mol. KCN	0.61
Second half-hour in 0.0001 mol. KCN	0.57
Third half-hour in 0.0001 mol. KCN	0.43

Experiment 18

Half-hour in normal sea-water	0.78
First half-hour in 0.0002 mol. KCN	0.17
Second half-hour in 0.0002 mol. KCN	0.19
Third half-hour in 0.0002 mol. KCN	0.21

*Experiment 19**

Half-hour in normal sea-water	1.63
First half-hour in 0.0002 mol. KCN	0.46
Second half-hour in 0.0002 mol. KCN	0.21
Third half-hour in 0.0002 mol. KCN	0.13

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*Experiment 20**

	OXYGEN CONSUMED cc.
Half-hour in normal sea-water	1.87
First half-hour in 0.001 mol. KCN	0.48
Second half-hour in 0.001 mol. KCN	0.16
Third half-hour in 0.001 mol. KCN	0.11

*Experiment 21**

Half-hour in normal sea-water	1.27
First half-hour in 0.001 mol. KCN	0.26
Second half-hour in 0.001 mol. KCN	0.22
Third half-hour in 0.001 mol. KCN	0.01

CONCLUSIONS

The following conclusions may be drawn from these experiments.

1. Very dilute concentrations of potassium cyanide increase the rate of oxygen consumption. This appears in experiments 5 and 11.

2. Slightly stronger concentrations, as 0.00001 and 0.00002 mol. have primarily a stimulating effect during the first half hour but later a depressing effect on the rate of oxygen consumption appears. This is the case in experiments 3, 6, 8, 12, 13, and 9.

3. In the stronger concentrations, 0.00004 to 0.001, the oxygen consumption is decreased throughout the entire period during which the animal was kept in the cyanide-containing sea-water. The decrease in oxygen consumption is very marked in the strongest concentrations used. Experiments 7, 14, 15, 2, 10, 17, 18, 19, 20, 21.

4. The effect of the cyanide varies with individual sponges. Thus the concentration 0.00004 may cause a primary stimulating effect followed by a depression in the case of one sponge, as in experiment 9, while in another sponge the same concentration shows only a depressing effect, as in experiment 14.

Attention may be called to the experiments which are starred, 13, 15, 17, 19, 20, and 21. These were performed upon the same individual sponge, the largest which I had. This sponge gave a high and fairly constant rate of normal oxygen consumption, and shows clearly the increasing depression accompanying increasing concentration of cyanide.

SUMMARY

The primary effect of potassium cyanide is a stimulation of the rate of oxygen consumption; this is followed by a depression of oxygen consumption which is the more marked the greater the concentration of

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the cyanide used. Only in very dilute concentrations is the stimulating effect demonstrable; in stronger concentrations it is masked by the rapidly ensuing depression.

The use of potassium cyanide as an agent for depressing the oxidation processes is therefore entirely justified, provided the proper concentrations are employed.

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INCREASE OF PERMEABILITY TO WATER FOLLOWING NORMAL AND ARTIFICIAL ACTIVATION IN SEA-URCHIN EGGS

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INTRODUCTORY

There are now many indications that the critical or initatory event in the activation of the resting egg is a temporary increase in the permeability of the protoplasmic surface-layer (or plasma-membrane) to water-soluble and diffusible substances. The precise connection between this variation of permeability and the total activation-process remains, however, to be determined. It is evident that in a system so complex as the egg-cell the immediate consequences of even so apparently simple a change may be various. The conditions of material interchange between egg and medium are altered; soluble substances, including possibly oxygen and carbon dioxide, enter and leave the egg more freely; there is also undoubtedly involved a change in the electrical polarization of the cell-surface, since the concentration and nature of the electrolytes on the two sides of the plasma membrane are altered. Only experimental evidence can decide finally as to the relative importance of these various possibilities. It seems likely, however, that the electrical change is itself the critical one. Electrical variations, due apparently to surface-changes, are well known to accompany stimulation and other cell-activities; and the general analogy between the initiation of division in resting egg-cells, and the excitation-process in irritable tissues, suggests that in the egg, as in the muscle-cell or nerve fiber, it is the electrical variation—and not the increase of permeability as such—which is the essential factor. External electrical currents stimulate muscles to contract; they may also activate the resting egg-cell in certain animals.[1]

[1] *Cf.* Schücking: Arch. f. d. ges. Physiol., 1903, xcvii, 86; Delage: Arch. d. Zoöl. expér. et gén., Ser. 4, ix, xxx; McClendon: this Journal, 1912, xxix, 299.

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According to this view, activation as well as stimulation depends primarily on electrical conditions.[2] In most cases of activation, however, the change of permeability would appear to precede and determine the electrical change. We can thus understand how cytolytic substances, heat, mechanical injury to the cell-surface, as well as the electrical current, may induce either activation of the resting egg or stimulation. Cytolytic substances, which in general are parthenogenetic agents as Loeb has shown, have as a class a depolarizing effect on the cell-surface, as indicated by the currents of injury which they produce in muscle. Other activating agents (heat, mechanical conditions) cause similar effects. The chief facts showing a general connection between increase of permeability and activation are: (1) the increase of electrical conductivity following normal or parthenogenetic fertilization,[3] (2) the readier entrance of substances like alkali and dyes into the egg immediately after fertilization,[4] (3) the loss or secretion of materials from the egg soon after the entrance of the spermatozoon,[5] (4) the fact that pure isotonic solutions of neutral salts (NaCl, $NaNO_3$, NaI, KCN, etc.) which initiate cleavage in sea-urchin and starfish eggs, lose their activating power in the presence of calcium or magnesium salts or anaesthetics, which prevent their permeability-increasing action.[6] Facts of this kind have a more than special interest, since

[2] In several earlier papers I have urged the importance of the bioelectric processes in the activation of the resting egg and in cell-division: *cf.* Biol. Bull., 1909, xvii, 202 *seq.*; this Journal, 1910, xxvi, 116; Journ. Exper. Zoöl., 1913, xv, 25 *seq.* Bataillon (Arch. de. zoöl. expér. et gén., Ser. 5, 1910, vi, 128) also regards the electric variation as probably an important factor in activation.

[3] McClendon: this Journal, 1910, xxvii, 240; Gray: Journ. Mar. Biol. Assoc., 1913, x, 50.

[4] *Cf.* E. N. Harvey: Science, N. S., 1910, xxxii. 565; Lyon and Shackell: *ibid.*, 249.

[5] *E.g.*, exit of pigment in Arbacia eggs (Lyon and Shackell, *loc. cit.*); of other diffusible substances, probably chiefly salts, from Arbacia eggs (McClendon, *loc. cit.*, p. 264); similar phenomena in frogs' eggs (Backmann and Runnström: Arch. f. d. g. Physiol., 1912, cxliv, 287). Phenomena of secretion are widespread; the separation of the fertilization-membrane probably belongs essentially in this category; decrease of volume with separation of materials from the egg is shown in vertebrate eggs (amphibia, fishes: *cf.* Bataillon, *loc. cit.*), and in many invertebrate eggs. The annelid egg affords striking instances, *e.g.*, Nereis (*cf.* F. R. Lillie: Journ. of Exper. Zoöl., 1912, xii, 414). The increased loss of catalase from the sea-urchin egg after fertilization, as observed by Lyon (this Journal, 1909, xxv, 199 , is also probably a consequence of increased permeability.

[6] *Cf.* my papers in this Journal, 1911, xxvii, 289, and Journ. Exper. Zoöl., 1914, xvi, 591.

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there is now little doubt that changes in the permeability and electrical polarization of the plasma-membrane constitute important controlling factors in a large variety of cell-processes. It is, therefore, desirable that our knowledge of these functional changes of permeability[7] should be extended in as many directions as possible.

During the last summer I have obtained definite evidence that the permeability of the egg to *water* undergoes marked increase immediately after fertilization. The entrance of water under the influence of osmotic pressure is several times more rapid in the fertilized than in the unfertilized egg. This may be shown readily as follows: Arbacia eggs are fertilized, preferably with the minimal quantity of spermatozoa, washed thoroughly with sea-water so as to remove the excess of sperm, and mixed with an equal quantity of unfertilized eggs. The mixed eggs are then transferred to hypotonic sea-water (*e.g.*, 40 volumes sea-water *plus* 60 tap-water), and examined at once under a low power. Within two or three minutes a well-marked difference in the size of the two kinds of eggs is apparent, the fertilized eggs being decidedly the larger. Measurement shows that after three minutes in the hypotonic medium the average diameter of unfertilized eggs is *ca.* 78 to 79 μ, that of fertilized eggs *ca.* 85 to 86 μ. Since the average diameter of the unaltered eggs in normal sea water is *ca.* 74 μ, the actual quantity of water entering the eggs in this interval of time may be estimated on the assumption that the eggs are spherical (volume equal to $\frac{4}{3}\pi r^3$). The volume of the normal egg of 74 μ is *ca.* 21.3 $\times$ 10^5 μ^3; that of the unfertilized water-distended egg of 78 μ is *ca.* 24.9 $\times$ 10^5 μ^3; of the fertilized water-distended egg of 85 μ is *ca.* 32.2 $\times$ 10^5 μ^3. The volume of water entering the unfertilized egg in three minutes is thus *ca.* 3.6 $\times$ 10^5 μ^3, that entering the fertilized egg is *ca.* 11 $\times$ 10^5 μ^3. This difference does not correspond exactly to the difference in permeability to water, as will be seen more fully below, but it illustrates clearly the greater readiness with which water enters the egg after fertilization. With a view to obtaining a more definite numerical estimate of the relative permeability of fertilized and unfertilized eggs, I have made a large number of measurements of the diameters of such eggs (and also of eggs with artificial fertilization-membranes) at different intervals after placing in dilute sea-water. From these measurements the rate of entrance of water—*i.e.*, of change of volume—can be determined.

[7] A general account of these phenomena is given in my paper in the Biol. Bull., *loc. cit.*, 199. *Cf.* also Bayliss: Principles of General Physiol., 1915, 124.

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MEASUREMENTS OF EGGS IN NORMAL AND HYPOTONIC SEA-WATER

The diameters of the eggs were measured under a magnification of 480 diameters with an ocular micrometer, the divisions of which were standardized by a Zeiss stage-micrometer. The sea-water containing the eggs was mounted on an ordinary microscopic slide, which was fastened in a mechanical stage so as to admit of ready and accurate placing of the image of the egg over the scale. No cover glass was used; any distortion of the eggs by compression was thus avoided; the objective was immersed in the sea-water. Each division of the ocular micrometer corresponded to *ca.* 3.5 μ. The image of the egg is sufficiently sharp to admit of measurement within a fifth of a scale division. In transforming the readings to microns only the first decimal is to be regarded as significant.

As a rule Arbacia eggs are almost perfectly spherical in form; occasionally they are slightly ovoid. Only round eggs were chosen for measurement; otherwise the eggs were measured at random as they came into the field. A circular optical section is of course not a proof of spherical form, but evident departures from sphericity are not frequent in these eggs. Fertilized eggs, and especially eggs with artificial fertilization-membranes, are more likely than unfertilized eggs to become oval or otherwise deformed after some minutes in dilute sea-water. It is therefore especially important in measuring such eggs to choose those having a circular optical image.

Measurements in normal sea-water. The average diameter of 58 unfertilized eggs was 74.1 μ, with extreme variants of 71.9 μ and 75.7 μ. Fertilization appears to be followed by a slight decrease in volume,[8] due probably to secretion of a part of the cortical substance; 14 fertilized eggs measured within ten minutes after fertilization, showed an average diameter of 73.2 μ.

Measurements in dilute sea-water. All of the following measurements were made in a mixture of 60 volumes tap-water plus 40 volumes sea-water. In this medium (with an osmotic pressure of *ca.* 11 atmospheres) the eggs swell considerably, but osmotic disruption—when it occurs—does not usually take place for ten minutes or more. There is a striking difference between fertilized and unfertilized eggs in their resistance to this change. Of the two the fertilized eggs are much less readily broken down by osmotic distention;[9] in unfertilized eggs the entrance

[8] *Cf.* Otto Glaser: Biol. Bull., 1914, xxvi, 84.

[9] That is, previously to the appearance of the cleavage-furrow; at this time the membrane undergoes a marked change in its properties and is readily destroyed by osmotic distention.

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of water is more gradual, but cytolysis always begins in a considerable proportion within fifteen minutes or even less in the solution, *i.e.*, some time before osmotic equilibrium is reached, and usually the majority of eggs are completely broken down within an hour. In the case of fertilized eggs water enters rapidly, osmotic equilibrium being usually reached within eight minutes or less, and the eggs remain intact without loss of pigment for several hours.

The volume of the eggs when osmotic equilibrium is reached is slightly less than double the volume in sea-water; the normal volume of the fertilized egg is *ca.* 20.6×10^5 cubic μ; in the above medium, after equilibrium is reached, it is *ca.* 40.4×10^5 cubic μ. If the egg were a perfect osmotic system (simple solution separated from external medium by a semi-permeable membrane) its volume would be inversely proportional to the osmotic pressure of the external medium; the sea-urchin egg approaches this condition closely enough to show that its behavior is essentially the same as that of an osmometer. It is to be expected that the egg will exhibit a smaller increment of volume in the hypotonic medium than would be the case with the ideal osmotic system, since a part of its volume consists of structural material, which occupies space but does not act osmotically.

The following tables (I, II) give measurements of the diameters of single eggs taken at regular intervals of one minute, begining one minute after placing in dilute sea-water. The procedure is simple; the normal eggs are kept in sea-water in finger-bowls; in each experiment the sea-water is first removed as far as possible, and a large volume (250 cc.) of the dilute sea-water is then added. The eggs are then mounted on the slide, and a single egg is chosen for observation; the necessary manipulation and the placing of the egg in position over the scale occupy some time, so that the first measurement cannot well be made sooner than one minute after placing in the dilute sea-water. Usually two eggs were kept under observation, and measurements were made, alternating from one to the other, at half-minute intervals.

Table I gives the diameters of three unfertilized eggs, from three different lots, measured at regular intervals of one minute up to fifteen minutes.

The degree and rate of water-intake shown by these measurements are typical. Seventeen similar measurements were made with unfertilized eggs; in six of these the eggs underwent cytolysis within eleven minutes or less; in nine cases regular measurements were made up to thirteen minutes or longer. In every case the egg showed the same

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TABLE I

Diameters of unfertilized eggs in μ, measured at one minute intervals after placing in dilute sea-water (60 volumes tap-water plus 40 sea-water). (Average diameter of unfertilized eggs in sea-water, 74.1 μ)

LOT FROM WHICH EGGS WERE TAKEN	MINUTES AFTER PLACING IN DILUTE SEA-WATER														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A. (Sept. 1)	77.5	78.8	80.3	81.7	82.7	83.5	84.5	85.0	85.5	85.9	86.4	87.1	87.8	88.0	88.0
B. (Sept. 2)	75.7	77.4	78.8	79.3	82.0	83.8	84.5	84.8	85.2	85.5	86.2	86.9	88.0	88.5	89.0
D. (Sept. 7)	76.4	77.8	79.6	81.7	83.1	84.5	85.1	85.9	86.9	88.0	88.4	88.7	89.1	89.6	90.1

behavior; water enters gradually at a rate which changes only slightly during the first few minutes; and later falls off by degrees as the station of equilibrium is approached. Typically equilibrium is not yet reached by fifteen minutes, as the course of the curve indicates (fig. 1). Measurements of eggs taken from the general dish of dilute sea-water show that the intake of water continues for twenty minutes or more. The average diameter of eleven unfertilized eggs taken from different lots, measured after about eighteen minutes in dilute sea-water, was 89.9 μ.

Under the same conditions the initial entrance of water in fertilized eggs is several times more rapid than in unfertilized eggs, and osmotic equilibrium is reached within nine or ten minutes or even less. The same rapid rate of entrance is seen in eggs in which artificial fertilization-membranes have been formed by butyric acid (see curves, figs. 1 and 2). Table II gives measurements similar to those of Table I

TABLE II

A. Diameters of eggs fertilized with sperm ten to twenty minutes before placing in dilute sea-water. (Average diameter of fertilized eggs in sea-water, 75.8 μ)

EGGS FROM LOT	MINUTES AFTER PLACING IN DILUTE SEA-WATER												
	1	2	3	4	5	6	7	8	9	10	11	12	13
A. (Sept. 1)	82.7	84.5	86.2	88.0	89.2	89.8	89.9	90.8	91.5	91.5	91.9	91.7	91.9
B. (Sept. 2)	80.3	82.1	86.2	88.4	89.0	89.8	90.8	91.5	92.0	92.0	92.0	92.0	
C. (Sept. 3)	81.7	85.2	88.0	89.1	90.1	91.5	92.2	92.2	92.2	92.2	92.2		

B. Diameters of eggs in which artificial membranes were formed with n/60 butyric acid twenty to thirty minutes before placing in dilute sea-water.

D. (Sept. 7)	80.3	84.5	86.2	88.0	89.0	90.5	91.2	91.5	91.9	91.9	91.9	91.9	
E. (Sept. 7)	81.0	84.5	86.6	88.0	89.0	89.8	90.8	91.5	91.5	91.5	91.5	91.5	
F. (Sept. 7)	78.1	81.1	82.7	84.1	85.0	85.9	87.1	88.0	88.0	88.0	88.4	88.7	

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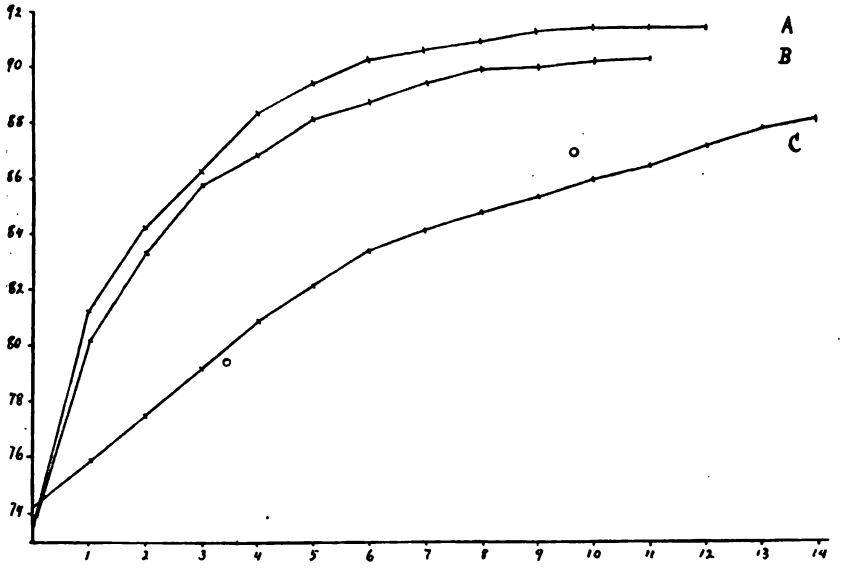


Fig. 1. Diameters of Arbacia eggs at different intervals after placing in dilute sea water. Ordinates are diameters in μ, abscissae minutes after placing in the hypotonic medium. The curves are not smoothed; the intersection-points of the co-ordinates are joined by straight lines. *A*, fertilized eggs; *B*, eggs with artificial membranes; *C*, unfertilized eggs.

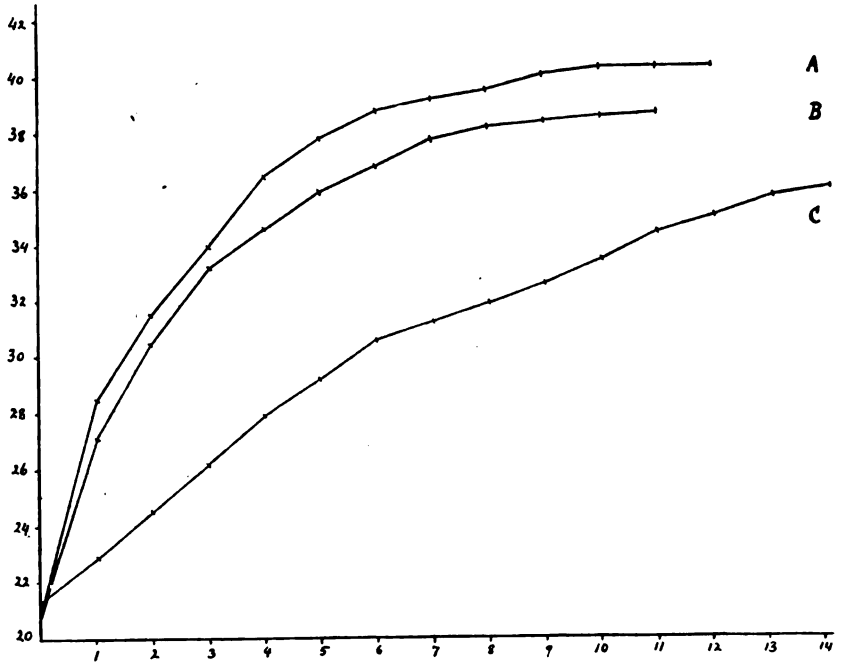


Fig. 2. Volumes of Arbacia eggs at different intervals after placing in dilute sea water. Ordinates are volumes (unit = 10^5 cubic μ); otherwise like figure 1.

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for sperm-fertilized eggs and eggs with artificial fertilization-membranes. Two of the fertilized eggs and one of the eggs with artificial membranes came from the same lots (A, B, and D) as the unfertilized eggs of Table I.

The differences between the three kinds of eggs are indicated most clearly by the curves (figs. 1 and 2). The figures from which these curves are constructed represent the averages of a number of observations made on eggs from different lots. These averages are given in

TABLE III

Diameters and volumes of eggs at different times after placing in dilute sea-water

TIME	UNFERTILIZED (AVERAGE OF 4 EGGS)		FERTILIZED (AVERAGE OF 6 EGGS)		ARTIFICIAL MEMBRANES (AVERAGE OF 6 EGGS)	
	Diameters	Volumes (unit = 10^6 cubic μ)	Diameters	Volumes (10^6 cubic μ)	Diameters	Volumes (10^6 cubic μ)
minutes	μ		μ		μ	
0	74.1	21.3	73.2	20.6	73.2	20.6
1	75.9	22.9	81.3	28.4	80.2	27.1
2	77.5	24.5	84.3	31.6	83.3	30.5
3	79.2	26.1	86.3	33.9	85.8	33.2
4	80.9	27.9	88.5	36.5	86.9	34.6
5	82.2	29.2	89.5	37.8	88.2	36.0
6	83.5	30.7	90.4	38.8	88.8	36.8
7	84.2	31.3	90.7	39.3	89.5	37.8
8	84.8	32.0	91.0	39.6	90.0	38.3
9	85.4	32.7	91.4	40.1	90.1	38.5
10	86.1	33.6	91.5	40.35	90.3	38.7
11	86.6	34.6	91.5	40.35	90.4	38.8
12	87.3	35.1	91.5	40.35		
13	88.0	35.8	91.5			
14	88.4	36.3				
15	89.2	37.3				

Table III; this table includes the diameters of the eggs at the different intervals after placing in the dilute sea-water, and also the volumes (calculated on the assumption that the egg is spherical).

According to these measurements nearly five times as much water enters the fertilized as the unfertilized egg during the first minute after placing in the dilute sea-water, and nearly four times as much during the first two minutes. This difference, however, is not an exact measure of the initial difference in permeability to water, for the reason that the rate of entrance is not uniform, but falls off in a curve which

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theoretically should have an exponential form, and more rapidly in the fertilized than in the unfertilized egg. What is required is the relative rates of entrance $\left(\frac{dv}{dt}\right)$ under the same conditions of osmotic pressure and area of membrane. From the above measurements, however, it is seen that during the first minute the fertilized egg takes up a volume of water of *ca.* 780,000 cubic μ, equal to 38 per cent of its initial volume; the egg with artificial membrane behaves similarly (*ca.* 700,000 cubic μ); while the unfertilized egg takes up only *ca.* 160,000 cubic μ,—*ca.* 7.5 per cent of its volume. This shows a somewhat remarkable resistance to the entrance of water, especially in the unfertilized egg. The surface-area of the Arbacia egg in sea-water is in round numbers *ca.* 17,000 square μ, and at the end of the first minute in dilute sea-water *ca.* 18,000 square μ. During the first minute therefore the volume of water transported across each square μ of surface is *ca.* 44 cubic μ for the fertilized egg, *ca.* 40 cubic μ for the egg with artificial membrane, and *ca.* 9 cubic μ for the unfertilized egg. This is with an initial driving force of *ca.* 13 atmospheres.[10] Later the difference between the rates of entrance is of course less; the slopes of the three curves indicate, as we should expect, that at osmotic equilibrium all eggs would have approximately the same volume,—about double that in normal sea-water; as already mentioned, however, unfertilized eggs frequently break down before this volume is reached.

The following table gives the observed average intake of water into the three kinds of eggs during the first ten minutes of immersion in dilute sea-water. It will be observed that in the unfertilized egg the average rate of entrance remains almost constant during the first six or seven minutes, but that in the other two it falls off rapidly from the

[10] The resistance encountered by water in penetrating the plasma membrane may be better appreciated if the facts are expressed in different units. One square centimeter = 10^8 square μ; one cubic centimeter = 10^{12} cubic μ; 9 cubic μ per square μ per minute is thus equivalent to 9×10^8 cubic μ per square centimeter per minute, or 0.0009 cubic cm. per minute. *I.e.*, approximately one cubic millimeter of water per minute per square centimeter of membrane, with a driving force of eleven atmospheres.

Harvey has remarked (*loc. cit.*, 565) upon the slowness with which Arbacia eggs swell in distilled water as indicating a surprising resistance to entrance of water. It is, however, clear that the surface of living cells must be highly resistent to the *solvent* action of water, and this implies resistance to its penetration. It would seem as if a membrane, in order to be truly semi-permeable, must have a limited penetrability to the solvent as well as to the solute. See below, p. 265.

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first. This is a further indication of the difference in the rate at which water enters. The rate of entrance declines with the decline in the gradient of osmotic pressure between egg-contents and medium; this gradient decreases relatively gradually with the less permeable eggs. The curves (fig. 3) bring out this relation in graphic form.

TABLE IV

Average intake of water per minute in eggs (unit = 10^5 cubic μ)

PERIOD	FERTILIZED		UNFERTILIZED		ARTIFICIAL MEMBRANES	
	Total intake	Average per minute	Total intake	Average per minute	Total intake	Average per minute
minutes						
0–1	7.8	7.8	1.6	1.6	6.5	6.5
0–2	11.0	5.5	3.2	1.6	9.9	5.0
0–3	13.3	4.4	4.8	1.6	12.6	4.2
0–4	15.9	4.0	6.6	1.6	14.0	3.5
0–5	17.2	3.4	7.9	1.6	15.4	3.1
0–6	18.2	3.0	9.4	1.6	16.2	2.7
0–7	18.7	2.7	10.0	1.4	17.2	2.5
0–8	19.0	2.4	10.7	1.3	17.7	2.2
0–9	19.5	2.2	11.4	1.3	17.9	2.0
0–10	19.75	2.0	12.3	1.2	18.1	1.8
0–11			13.3	1.2		
0–12			13.8	1.15		
0–13			14.5	1.1		
0–14			15.0	1.1		

A more exact measure of the relative permeabilities is furnished by the relative times required for the entrance of the same volume of water. It will be seen from Table III that almost as much water enters the fertilized egg in one minute as enters the unfertilized egg in five; the egg with artificial membrane is only slightly less permeable than the fertilized egg. According to this comparison the plasma-membrane of the fertilized egg is from four to five times more permeable to water than that of the unfertilized egg. This conclusion is confirmed by the results of the analytical treatment which follows.

The rate at which water enters the egg—*i.e.*, the volume traversing the membrane in unit time,—is determined by several factors. Of these the chief are: (1) the driving force of osmosis; this (assuming that the membrane is semi-permeable) is at any time equal to the difference between the internal and the external osmotic pressures

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(*i.e.*, *ca.* 13 atmospheres at first), diminished by the opposing forces of cohesion or elasticity of the egg-substance and the surface-tension; (2) the frictional resistance to the passage of water through the membrane (the reciprocal of the permeability to water);[11] and (3) the area of the membrane, *i.e.*, of the egg-surface. Two of these factors, (1) and (3), vary continuously as water enters and the volume of the egg increases; probably also the permeability of the membrane varies somewhat as its area is enlarged, but the comparatively regular course of the curve in the fertilized eggs indicates that during the period of

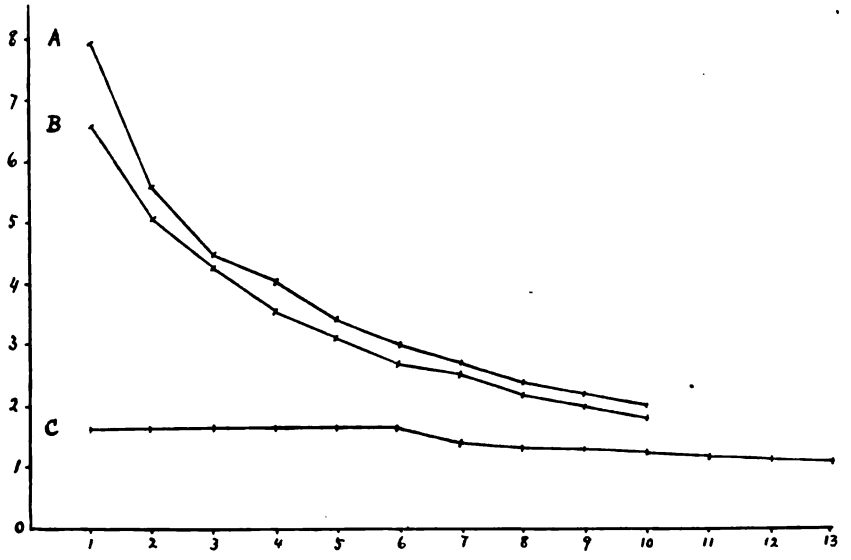


Fig. 3. Average intake of water per minute during immersion in dilute sea water for varying periods. Ordinates are volumes (unit = 10^5 cubic μ); abscissae, total time of exposure to dilute sea-water.

observation this change is relatively slight; in the unfertilized eggs the curve is less regular and its course for the first six or seven minutes is nearly straight; this appears to indicate that the decrease in the osmotic driving force during this period is nearly balanced by a slight though progressive falling off in the resistance to entrance to water. The calculated permeability-constant K does increase somewhat

[11] For a discussion of the rôle played by this factor in osmotic phenomena in general, *cf.* Antropoff: Zeitschr. f. physik. Chem., 1911, lxxvi, 721.

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during this period (see Table V). On the whole, however, the alterations of permeability do not appear to be great, at least during the first ten minutes of immersion; later, when the extension of the membrane becomes sufficient to alter its structure, the general permeability must increase rapidly, as shown by the loss of pigment and general disintegration. In the following calculation I shall for simplicity leave out of consideration the forces of elasticity, cohesion, and surface-tension—which are undoubtedly negligible in comparison with osmotic pressure—and also the change in the area of the membrane during the period of water-intake. A calculation of permeability-constants on the basis of the volume of water passing across the *unit area* of membrane in each of the times considered (instead of that entering the whole egg) would be theoretically more exact; but it introduces complexities of treatment which it seems best to avoid, and does not alter the result in any essential manner.

According to the above analysis of conditions the rate of entrance of water into the egg at any moment will be indicated by the equation:

$$\frac{dv}{dt} = q\sigma\,(P_e - P_m) + \text{const.} \qquad (1)$$

where v is the volume of water entering in the time t, q the area of the membrane, σ the permeability of the membrane to water (reciprocal of frictional resistance to its passage), P_e the osmotic pressure of the egg-contents, P_m that of the external medium. *Const.* indicates any other factors, *e.g.*, physiological, which are additive in their effect.

The above equation is similar in form to that representing the course of a monomolecular chemical reaction, in which, according to Guldberg and Waage's law, the rate of change is at any moment proportional to the concentration (= osmotic pressure) of the substance undergoing transformation—*i.e.*: $\frac{dc}{dt} = Kc$, of which the integration-form is: $k = \frac{1}{t} ln \frac{a}{a-x}$, a being the initial concentration of the substance, and $a-x$ the concentration at the end of the time t. Similarly, assuming that q and σ in the above equation remain essentially unchanged during the period under consideration, the rate of entrance of water into the egg is at any moment proportional to the osmotic driving force, $P_e - P_m$ —*i.e.*:

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$$\frac{dv}{dt} = K\,(P_e - P_m) + \text{const.} \tag{2}$$

where K is the constant indicating the rate of entrance of water into the egg under a definite pressure. This constant may be called the "permeability-constant." The integrated form will be:

$$K = \frac{1}{t}\, ln\, \frac{P_e - P_m}{P_t - P_m} \tag{3}$$

where P_e is the osmotic pressure within the egg at the beginning of the experiment (= the osmotic pressure of sea-water), P_m that of the external medium, and P_t the osmotic pressure within the egg at the end of the time t. It is evident that since the egg is originally in osmotic equilibrium with sea-water, the osmotic driving force at the outset of the experiment is the same as the difference between the osmotic pressures of sea-water and external medium. This osmotic force is $P_e - P_m$; after the time t, when the pressure within the egg has fallen to P_t, the osmotic force becomes $P_t - P_m$.

In order to apply this formula to the above observations it is necessary to translate the terms of osmotic pressure into those of volume. The measurements given in Table III show that the volume of the egg increases steadily, after placing in dilute sea-water, until osmotic equilibrium is reached; then the osmotic pressure inside the egg is equal to that outside. They also show that the ratio of the final to the initial volume is approximately the same as that of the initial to the final osmotic pressures within the egg (= the ratio of the osmotic pressures of sea-water and external medium); in other words, the volume of the egg, in a medium of about half the original osmotic pressure, is approximately doubled. If we regard the egg as a perfect osmotic system (with volumes the reciprocals of osmotic pressures), we may substitute volumes for pressures in the above equation; we may then compare the theoretical with the observed volumes. The equation then takes the form:

$$K = \frac{1}{t}\, ln\, \frac{V_{eq} - V_o}{V_{eq} - V_t} \tag{4}$$

Where V_{eq} is the volume of the egg at osmotic equilibrium in dilute sea-water, V_o its volume at the beginning of the experiment (*i.e.*, its volume in sea-water), and V_t its volume at the end of the time t. $V_{eq} - V_o$ thus represents the value of the osmotic driving force at the

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beginning of the experiment, and $V_{eq} - V_t$ its value at the end of the time t.[12]

If the entrance of water in the foregoing experiments is in fact mainly determined by osmotic conditions, the value of K as deduced by this formula from the above observations should be approximately constant. In the following table K is evaluated for each of the three kinds of egg, substituting for V_{eq}, V_o, and V_t the volumes given in Table III. The final volume V_{eq} is regarded the same in all eggs, namely 40.4×10^5 cubic μ; the initial volume of the unfertilized egg is 21.3×10^5 cubic μ; that of the eggs with artificial membrane is assumed to be the same as that of the fertilized eggs (20.6×10^5 cubic μ). In the evaluation of K common logarithms are used.

In the unfertilized eggs K shows relatively little variation during the first fourteen minutes in dilute sea-water. In the fertilized egg the entrance of water during the first minute is always relatively large (See Table II). This is probably the expression of a disturbance (analogous to osmotic stimulation) due to sudden transfer to the dilute medium. During the next seven minutes K shows only minor fluctuations about a mean value which is about four times that of the unfertilized eggs. The approximate constancy of K shows that the entrance of water into the egg in dilute sea-water is determined by purely osmotic conditions. In the eggs with artificial membranes K is also three or four times greater than in the unfertilized eggs, and the initial rate of entrance is higher than later; in these eggs, however, K is not constant but shows a steady decline. This feature is interesting, since it indicates an imperfect semi-permeability and an inability of the membrane to resist extension; probably it is to be correlated with the fact that the condition of such eggs is unstable; normally

[12] The general solution of a problem of this kind, as given to me by Prof. A. G. Webster, is as follows:

$$V_t = V_o + (V_{eq} - V_o)\ (1 - e^{-kt});\ \text{hence:} \tag{1}$$

$$\frac{V_t - V_o}{V_{eq} - V_o} = 1 - e^{-kt};\ \text{that is:} \tag{2}$$

$$e^{-kt} = 1 - \frac{V_t - V_o}{V_{eq} - V_o} = \frac{V_{eq} - V_t}{V_{eq} - V_o};\ \text{or expressed in logarithms,} \tag{3}$$

$$-k = \frac{1}{t} \log \frac{V_{eq} - V_t}{V_{eq} - V_o},\ \text{or}\ k = \frac{1}{t} \log \frac{V_{eq} - V_o}{V_{eq} - V_t}. \tag{4}$$

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TABLE V

A. Unfertilized eggs

(*Units of volume* = 10^6 *cubic* μ.) V_o (*initial volume*) = 21.3; V_{eq} (*final volume*) = 40.4; $V_{eq} - V_o = 19.1$

t (TIME IN MINUTES AFTER PLACING IN DILUTE SEA-WATER)	V_t (VOLUME AT TIME t)	$\frac{V_{eq} - V_o}{V_{eq} - V_t}$	$\frac{1}{t} \log \frac{V_{eq} - V_o}{V_{eq} - V_t} = K$
1	22.9	1.1	41
2	24.5	1.2	40
3	26.1	1.4	49
4	27.9	1.6	51
5	29.2	1.7	46
6	30.7	1.9	47
7	31.3	2.1	46
8	32.0	2.3	45
9	32.7	2.5	44
10	33.6	2.8	45
11	34.6	3.3	46
12	35.1	3.8	48
13	35.8	4.2	48
14	36.3	4.7	49
Average.....			46

B. Fertilized eggs

$V_o = 20.6$; $V_{eq} = 40.4$; $V_{eq} - V_o = 19.8$

1	28.4	1.6	204
2	31.6	2.25	171
3	33.9	3.0	159
4	36.5	5.1	177
5	37.8	7.6	176
6	38.8	12.4	182
7	39.3	18.0	179
8	39.6	27.4	174
9	40.1	66.0	202
Average.....			180

C. Eggs with artificial membranes

$V_o = 20.6$; $V_{eq} = 40.4$; $V_{eq} - V_o = 19.8$.

1	27.1	1.5	176
2	30.5	2.0	150
3	33.2	2.7	144
4	34.6	3.4	136
5	36.0	4.5	131
6	36.8	5.5	123
7	37.8	7.6	123
8	38.3	9.4	122
9	38.5	10.4	113
10	38.7	11.6	106
Average.....			132

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they break down in sea-water unless subjected to further treatment (*e.g.*, with hypertonic sea-water).

The plasma membrane of the unfertilized egg is thus characterized by a low permeability to water. During the early period of distention in dilute sea-water this condition shows little change; later the semi-permeability appears to break down suddenly, with a resulting disintegration of the egg. In consequence of fertilization the permeability rapidly increases to about four times that of the unfertilized egg; the formation of artificial fertilization-membranes has a similar effect upon the egg; but such eggs are less capable than normally fertilized eggs of resisting distension without alteration of the osmotic properties of the membrane.[13]

These facts suggest certain more general possibilities, to which brief allusion may be made here. If the permeability to water varies so widely in different functional conditions of the cell, it is probable that the permeability to other substances, which also apparently pass the membrane readily at all times, may similarly vary. The chief of these are carbon dioxide and oxygen; it is possible that under certain conditions the membrane may be relatively impermeable to such substances. The egg previously to fertilization appears to be enclosed by a membrane which is waterproof to a relatively high degree; and it seems probable that the isolation thus resulting may account in part for the low rate of metabolism.[14] The possibility that the per-

[13] It might be expected that the difference in the osmotic properties of the egg before and after fertilization would be associated with a difference in the physical consistency and other properties of the surface-film. Such a difference might be demonstrated by micro-dissection. Dr. Chambers, whom I have consulted on this point, informs me that the unfertilized egg is less easily cut or torn with a needle than the fertilized egg, and that the sides of the tear show a greater tendency to fuse. This difference of behavior is suggestive in relation to the foregoing observations.

It might be objected that these observations indicate merely a difference in the tenacity or extensibility of the membrane, and not in its permeability as such—just as (*e.g.*) a rubber net and a steel net are equally *permeable*, though unequally extensible. Water would enter more rapidly the egg with the more extensible membrane. But any change in consistency, and especially a change in density (*i.e.*, colloid content), would almost certainly involve a change in permeability; there is moreover good independent evidence of such a change (see introductory section). The present paper, however, is concerned not so much with the conditions as with the fact of increased permeability.

[14] Lyon and Shackell (*loc. cit.*) find, however, that unfertilized and fertilized eggs stained equally with methylene blue, and decolorized by reduction in a stream of hydrogen in an Engelmann chamber, regain the color, on readmission

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meability to carbon dioxide may vary is not to be summarily rejected simply on the ground of the lipoid-solubility of this compound. Such a phenomenon as the increased output of carbon dioxide in nerves during stimulation[15]—a change unaccompanied by heat-production[16]—may be simply an expression of increased permeability, rather than of increased formation of this compound within the cell.

It should also be noted that this impermeability to water argues a high degree of density and insolubility in the surface-film. These properties, however, are retained only during life; they must therefore be an expression of cell-metabolism. One fundamental feature of metabolism is that a variety of surface-active materials of low water-solubility are continually being produced; apparently these gather in the surface-film, and are as continually being disintegrated or removed, *e.g.*, by oxidation. Only on some such hypothesis can we understand the remarkable fact that living cells, despite the water-soluble nature of most of the substances composing them, and the large surface-area which they expose to the solvent action of the medium, do not undergo solution, but preserve their semi-permeability and other properties intact.[17]

of air, at about the same rate. They are inclined to interpret this observation as indicating an equal permeability to oxygen in both eggs. In view of the much higher rate of oxidation in the fertilized eggs, and the presence of substances in the protoplasm which compete with the methylene blue for oxygen, we should rather expect, if the permeabilities were equal, that the slowly oxidising unfertilized egg would decolorize *more* rapidly than the rapidly oxidising fertilized egg. The observation may thus indicate a relatively rapid entrance of oxygen into the fertilized egg; unfortunately it is not decisive either way.

[15] Tashiro: Amer. Journ. Physiol., 1913, xxxii, p. 107.

[16] A. V. Hill: Journ. of Physiol., 1912, xliii, p. 433.

[17] One of the observations of Lyon and Shackell has an interesting bearing on the present problem. They found that iodine is taken up much more rapidly by unfertilized than by fertilized eggs (*loc. cit.*). If, as they suggest, the iodine is disposed of by the lipoids of the plasma membrane, this fact would indicate a greater content of lipoid or fatty substances in the plasma membrane of the unfertilized egg. It is thus possible that a relation exists between iodine-combining power and impermeability. The impregnation of the membrane with certain fats or lipoids—*e.g.*, with an unsaturated compound like cholesterol, which is also water-insoluble—would increase at the same time both its iodine-combining power and its impermeability to water. If these are the actual conditions in the egg, it would appear that fertilization leads to an accelerated removal or destruction of such compounds.

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SUMMARY

1. The rate of entrance of water into fertilized Arbacia eggs in hypotonic sea-water of *ca.* 11 atmospheres osmotic pressure is approximately four times that into unfertilized eggs.

2. The rate of entrance at any time is determined by the osmotic pressure gradient (between egg and medium) prevailing at that time, and by the permeability of the plasma membrane to water. This permeability is therefore four times greater in the fertilized than in the unfertilized egg. The artificial formation of fertilization membranes (by butyric acid) is followed by a similar marked increase of permeability to water.

3. The osmotic properties of unfertilized and normally fertilized eggs remain approximately constant during the first eight or more minutes of immersion in dilute sea-water. On the other hand, eggs with artificial membranes show a progressive change in osmotic behavior, indicating probably a relatively unstable condition of the plasma membrane.

4. The essential constancy in the rate of entrance of water (relatively to the existing gradient of osmotic pressure) into fertilized and unfertilized eggs, during a period in which the water-content of the egg is almost doubled, shows that the difference between the two kinds of eggs is due not to a difference in the condition of the internal protoplasm, but simply to a difference in the resistance of the membrane to passage of water.

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CARDIODYNAMICS IN HEART BLOCK AS AFFECTED BY AURICULAR SYSTOLE, AURICULAR FIBRILLATION AND STIMULATION OF THE VAGUS NERVE

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The purpose of this research is to study the relation of ventricular efficiency to ventricular filling and to analyze and correlate the various effects of auricular contraction on cardiodynamics.

It is commonly stated that the ventricles tend to empty themselves with each ventricular systole regardless of the initial ventricular volume obtaining. In this statement the function of auricular systole is suggested, that is, auricular systole should increase ventricular output in direct proportion to the increased ventricular filling resulting therefrom. But this statement is an observation rather than an explanation and in addition needs some modification, for the reverse holds with equal force, that is, the greater the initial ventricular volume, the less perfectly do the ventricles empty themselves. Unless each statement is carefully modified it would be more accurate to state that increased ventricular filling, within certain limits, increases ventricular output. Even when initial volume is small the ventricles fail to empty themselves completely with each ventricular systole. In view of this fact it is very significant that increased volume should increase ventricular output at all. It proves that increased initial ventricular volume per se is not the factor determining ventricular output but rather the secondary conditions arising from the increased volume.

A sudden increase of ventricular volume as produced by auricular systole means an increased initial length of ventricular fiber, an increased initial intraventricular tension, and an enhancing surface-volume relation, i.e., a greater increase of ventricular volume than ventricular surface.

All these factors should work toward greater ventricular efficiency. They are suggested as a result of work on the turtle's auricles, the

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properties of which permit independent analysis of the factors of initial length of fiber and initial tension in relation to muscular contraction.[1]

We see from the foregoing that with other things constant, ventricular output does not necessarily vary in direct proportion to the degree of ventricular filling, and the reason is obvious. If ventricular output is not solely dependent on ventricular volume per se, which means that the ventricular muscle is not strong enough under all conditions to produce complete ventricular emptying, the increased ventricular output resulting from increased ventricular filling will depend on the degree to which the secondary enhancing factors are increased by increased volume.

With each auricular systole the enhancing effects of initial length, initial tension and surface-volume relation are increased simultaneously. The increased enhancing effect of each will depend on various conditions. In addition, these factors influence each other. It is difficult therefore to determine quantitatively the importance of each. All that can be hoped for is a general analysis of these factors, pointing out their interrelation and in a rough way their relative importance.

In a previous research on the mammalian heart,[2] in which the effectiveness of auricular contraction was varied, changes of venous and arterial pressures were used as indices of propulsion of blood by the ventricles, indicating whether the blood was accumulating on the venous or on the arterial side of the ventricles. Though changes of ventricular volume resulting from auricular systole and ventricular systole were not recorded, the enhancing effect of auricular systole on ventricular efficiency was ascribed in the main to the filling effect of auricular systole on the ventricles.

In the present research more definite information was obtained. The effects of auricular contraction were studied from a number of points of view. Records were made of auricular contraction, ventricular contraction, variations of length of ventricular fiber (which indirectly gives ventricular volume changes), intraventricular tension (which shows both initial and final tension), volume output, venous pressure and venous pulse.

To determine with any degree of exactness the relative importance of the many effects of auricular systole on ventricular efficiency, control of conditions and limitation of variable factors to a minimum is highly desirable.

[1] Gesell: This journal, 1916, xxxix, 239.
[2] Gesell: This journal, 1911, xxix, 32.

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The use of a modified heart-lung[3,4] preparation with the heart in block offers excellent opportunities for cardiodynamic study. In such experiments rate of auricular and ventricular contractions, nature of auricular contraction, time relation of auricular systole to ventricular systole, venous pressure and capillary resistance are all under perfect control. To simplify description, the apparatus employed in these modified heart-lung experiments will be described under three headings: (1) venous system; (2) arterial system; (3) pneumatic blood pump.

I. Venous System (see fig. 1). This system was devised to supply the heart with blood at any desired constant venous pressure. In the main it consists of an upper (4) and lower (7) reservoir, and overflow (8, 9 and 12) and heart flow (11 and 15). The blood in it takes the following course: reaching the upper reservoir (4) through tube (1) it passes to the lower reservoir (7). From there the blood may go either to the heart via the heart flow (11 and 15), or back to the pump via the overflow (8, 9 and 12). The lower reservoir is a specially constructed double boiler, fitted with an overflow (9) and a heart flow (11 and 15) The reservoir proper is fixed within the outer jacket (10) which is filled with water maintained at the proper temperature with burner (16). A funnel (11) passes from the bottom of the reservoir through the outer jacket and connects with the heart flow (15). The blood is filtered through glass wool in the funnel.

The flow of the blood from the upper to the lower reservoir is regulated to insure a continuous flow over the overflow cut (8), thereby giving a constant venous pressure. It will be noted that this cut is made to accommodate large overflows without raising appreciably the venous pressure. The small notch which prevents damming back of solution by surface tension serves the same purpose. The upper reservoir has two uses. It prevents formation of froth in the lower reservoir and insures a more uniform flow of blood into the lower reservoir than could be obtained directly from the pump. The gauze filter (5) on tube (1) serves the same purpose.

The magnitude of venous pressure is regulated by the adjustable stand (3). The entire venous system is attached to rod (2) and moves as a whole when the venous pressure is altered.

[3] Martin: Croonian Lecture, Phil. Trans. Roy. Soc. London, 1883, clxxiv, 663.
[4] Knowlton and Starling: Journ. Physiol., 1912, xliv, 206.

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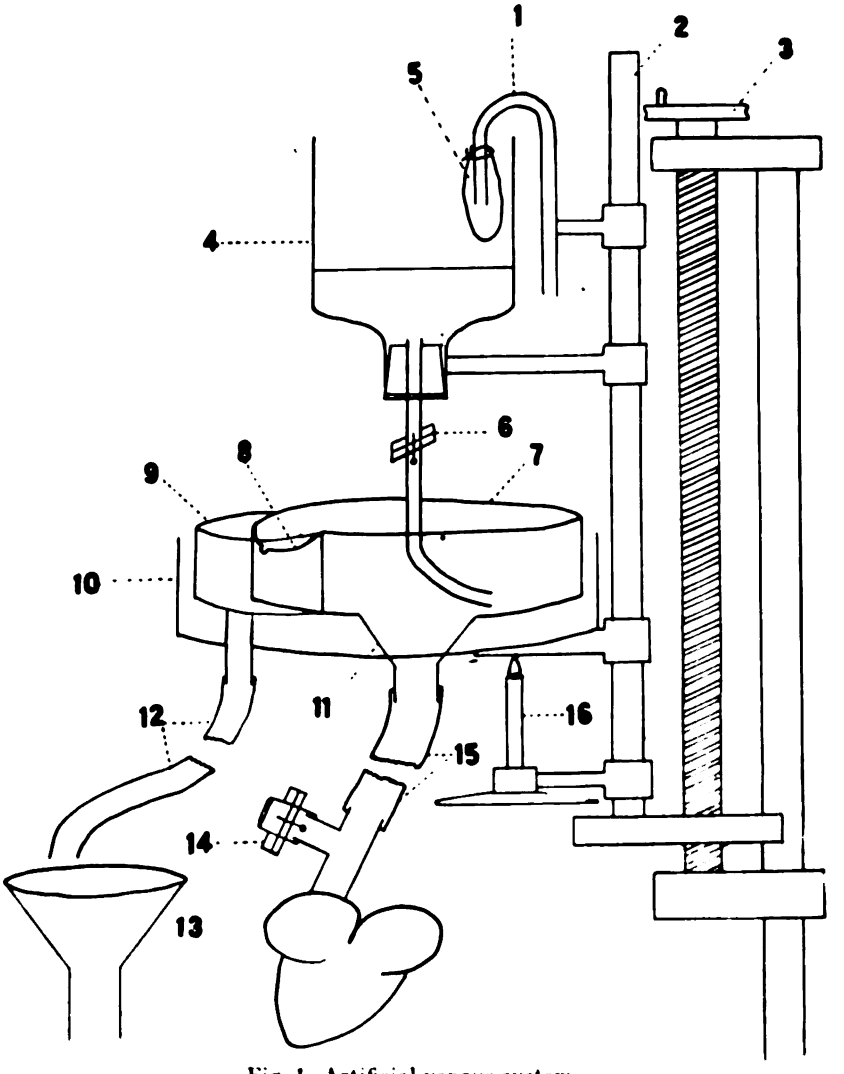


Fig. 1. Artificial venous system.

2. ARTERIAL SYSTEM (see fig. 2). This system is composed in the main of the capillary resistance (19 to 20), elasticity chamber (18), and differential volume flow recorder (34). As the blood comes from the heart it follows this route—through tube (17), into the capillary resistance (20), through (21 and 28) to the volume flow recorder (29,

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30 and 34) and from there into funnel (13) back to the pump. The arrangement of the capillary resistance in the large T Tube (19) is shown. The pressure exerted on the outer surface of membrane (20) is regulated by the three way stopcock (33) which connects with the source of air pressure (22). With the cock in the position shown, the air pressure is transmitted to tube (19) and read off on mercury manometer (24). Air chamber (25) permits easy regulation of the pressure applied on membrane (20). Through tube (23) which is open to the exterior, the pressure may be decreased.

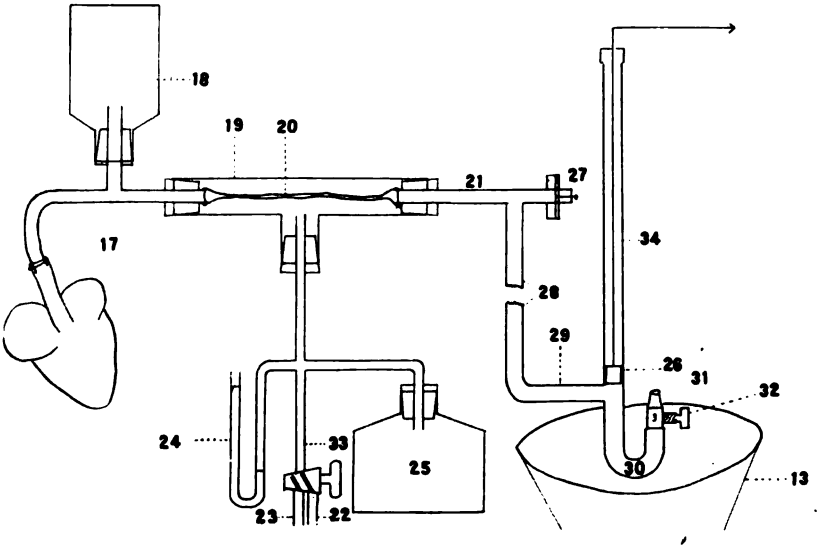


Fig. 2. Artificial arterial system.

Air chamber (18), as described by Knowlton and Starling, gives elasticity to the arterial system, permitting storage of ventricular energy.

A modified volume flow recorder similar to one previously described[5] was employed. It consists primarily of a three way connecting tube fitted with a graduated stopcock. The horizontal tube (29) is short and connects with the source of liquid to be measured. It joins at right angles a vertical tube (34) which contains a cork float and glass writing point (26). This tube connects with the U-tube (30) which is fitted with a graduated stopcock (32). The opening of this cock is on a level with the entering tube (29). This arrangement permits the

[5] Gesell: This journal, 1915, xxxviii, 402.

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recording of flows from zero to a flow dependent on the height of tube (34) and the degree to which the cock is opened. This cock is threaded, and the block so marked that the degree of opening within one-fourth of a turn is easily read. The smaller the opening the more delicate is the recorder. A position of the cock suitable for an entire experiment is quickly found. Should a change be necessary the cock may be set at any known position and the recorder calibrated at the close of the experiment for the positions employed.

This recorder has certain advantages over such recorders as the tipping bucket, intermittent siphons, etc. It gives a continuous record, and consequently is quicker in indicating the moment of changed volume flow. It shows the pulse and gives an index to the individual ventricular outputs.

3. Pneumatic Blood Pump (see fig. 3). This consists of a system of valves in connection with a Woulfe's bottle which has three openings —blood outlet (46), blood inlet (47), and air inlet (48). The motive power is pulsatile air pressure furnished by the rotating stop cock (39) connected with the source of air pressure (40). A brass tube (36) passes through the blood inlet (47). This tube is closed at the lower end and its walls perforated with a number of holes which are covered on the outside with gold beater's skin (37). This arrangement permits blood to enter but not to leave the bottle through (36) and (47). The source of blood for the pump is the overflow and volume flow (12) and (34) reaching the blood inlet by funnel (13) and tube (35). The outflow tube (46) is closed above and open below, and nearly reaches the bottom of the bottle. The upper half of the tube (43) is enclosed by a larger tube (42) and its walls perforated and covered with gold-beater's skin (45). This arrangement directs the flow from the bottle through tube (1) into the venous reservoir and prevents backflow into the bottle. The capacity of the pump with a given air pressure depends upon the position of cock (38) -that is on the relative amount of air escaping and entering the bottle. The position of this valve and the rate of revolution of valve (39) can be so regulated that the pressure in the Woulfe's bottle falls to zero between each pulsation, permitting a free inflow of blood through the blood inlet.

OPERATIVE PROCEDURE

Prior to the experiment a large dog was bled, and the circulatory system washed out with Ringer's solution, until the volume of diluted blood obtained from the animal amounted to 1500 cc. The blood was defibrinated.

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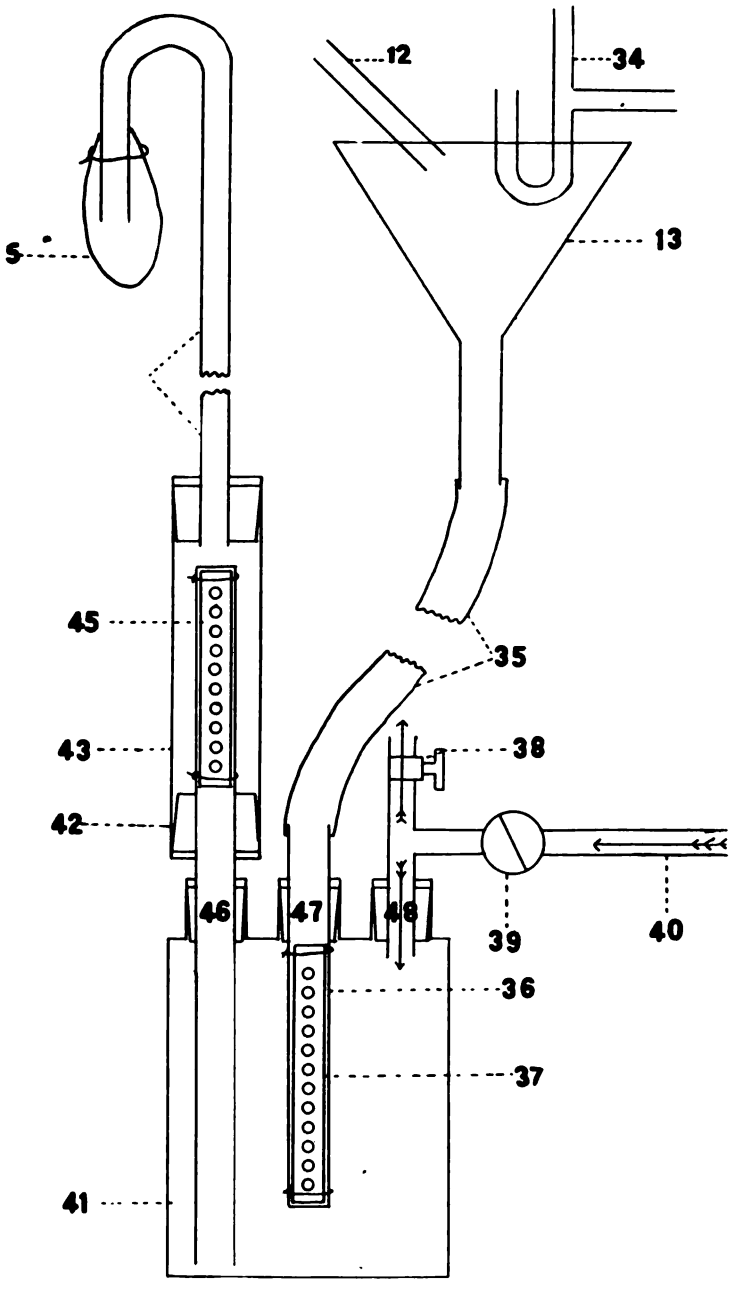


Fig. 3. Pneumatic blood pump.

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The animal to be experimented upon was then prepared. Morphin and ether were given. The heart was exposed, and artificial respiration administered. A large cannula was inserted in the common carotid artery which was obstructed below the point of insertion. All the arteries springing from the arch were ligated and a heavy ligature passed under the aorta (but not tied) just distal to the common carotid. A large cannula (14) was then inserted into the superior vena cava and a heavy ligature passed under the inferior vena cava. The auriculoventricular bundle was crushed with the Erlanger heart clamp, and the clamp removed when block was complete. Arrangements were then made to record either arterial or left intraventricular pressure, as this is a necessary index to the working condition of the heart in the heart-lung preparation. The defibrinated blood, at body temperature, was set into circulation through the pump and venous system preparatory to perfusion. The blood circulating in the animal was then drawn in the following way. The obstruction below the arterial cannula was removed, the heavy aortic ligature tied, and the blood allowed to pass without resistance through (20) and out at (27), figure 2. This blood was defibrinated and used in the experiment. When the animal was bled the ligature on the inferior vena cava was tied, and the blood from the venous reservoir allowed to enter the heart through the superior vena cava. The resistance in (20) was immediately increased to the desired level. As Starling points out, the nutrition of the heart depends on the resistance offered to the blood. The success of the experiment, I have found, depends on promptly supplying sufficient venous pressure and arterial resistance. When once started the experiment goes on automatically for hours, without any further attention.

Since the circulation is confined to the heart and lungs only, the ether was now withdrawn. The auricular and ventricular contractions were recorded by the suspension and air transmission method. Intraventricular and arterial pressures were recorded with the Hürthle manometer, venous pressures by water and membrane manometers. The venous pressures were taken from the side tube of cannula (14) figure 2.

Intraventricular pressure was recorded with the aid of a trocar cannula previously used but not described.[6] Cannulas somewhat similar have been devised[7,8] but since the trocar cannula here employed has certain points of structure which may prove valuable to others it is

[6] Gesell: This journal 1911, xxix, 32.
[7] Straub: Arch. f. d. ges. Physiol., 1911, cxliii, 69.
[8] Piper: Arch. f. Anat. u. Physiol., 1912, 343; 1913, 385.

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shown in figure 4 in its parts and assembled. It consists of a trocar (*A*), a cannula (*B*), a three way stopcock (*C*), and a sleeve with disc (*D*). The trocar (*A*) fits snugly in cannula (*B*) passing through and locking stopcock (*C*). The sleeve is threaded to fit the lower threaded end of the cannula. Before inserting and fixing the trocar cannula in position, it is assembled as shown with the sleeve turned high on the cannula. A purse string suture is stitched in the heart, and the trocar cannula passed through its center. The free ends of the suture are passed through a slit in the disc running from the periphery to the sleeve and tied about the sleeve. Holding sleeve and disc (*D*) the cannula is then turned so that the distance (*F*) equals approximately the thickness of the ventricular wall, insuring at all times the proper position of the intraventricular end of the cannula. Tube (*E*) is connected with the manometer, trocar (*A*) withdrawn to a marked point which unlocks the cock but still obstructs the upper end of the cannula; the cock is turned and the trocar completely withdrawn.

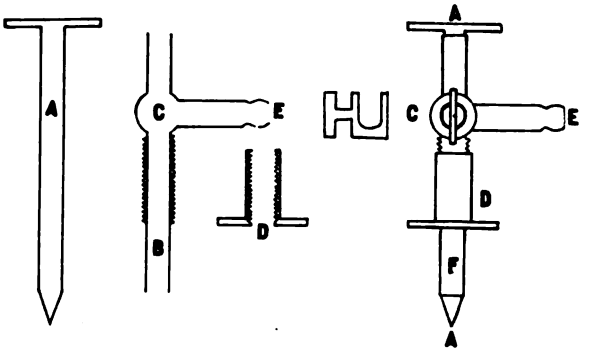


Fig. 4. Trocar cannula.

Changes in length of ventricular fiber were recorded and used as an index to ventricular volume changes, due to ventricular systole, filling action of venous pressure and auricular systole. The complexity of the experiment required a compact myocardiograph. The piston myocardiograph shown in figure 5 was employed. It consists of a cylinder and piston mounted on tubes (*A*), (*B*), and (*C*). The joint at (*A*) and (*B*) is welded; connecting (*A*) and (*C*) is a hinge joint permitting free movement in the plane of the piston stroke. The cylinder (*E*) is a thin turned brass tube, adjustable by block and set screw to any point on tube (*B*). Tube (*B*) is flattened on one side to fit a corresponding flattened surface on block (*F*), which prevents rotation of the cylinder on tube (*B*) thereby preventing binding of the piston. The piston myocardiograph is fastened in place by two threaded needles stitched into the heart, and the threads slipped under the needles and

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tied as shown. The needles are inserted any desired distance into the tubes (*C*) and (*B*), and fixed with set screws (*G*). The device may be suspended by a light spring or thread; the best place of suspending the whole from tube (*A*) is soon found and fixed with colophonium. Cylinder (*E*) is then connected through tube (*K*) by rubber tubing with a piston recorder which records the changes of length of ventricular fiber.

In devising this piston myocardiograph effort was made to procure lightness, compactness, free movement and easy adjustment. The instrument does not interfere with the action of the heart and offers no difficulties if the piston recorder is properly balanced and freely moveable.

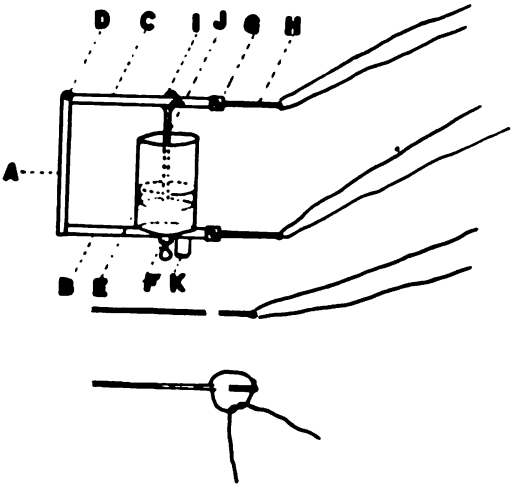


Fig. 5. Piston myocardiograph.

Time was marked in seconds and fifths of seconds.

The rate of ventricular contraction was controlled by unipolar stimulation selecting only the break shocks with a rotary stimulus selector. It was possible therefore, to study the effect of auricular systole on either slowly or rapidly beating ventricles.

The effects of auricular systole were studied by annulling or modifying the effectiveness of auricular contraction in various ways. In some cases the magnitude and rate of auricular contraction were controlled by faradic stimulation of the auricles and of the vagus nerve. In other cases the time relation of auricular to ventricular systole was changed, by the production of auriculo-ventricular interference waves (see fig. 6).

To produce these waves the ventricles are stimulated at approximately the rate of auricular contraction. The more closely the two rates are approximated the greater the number of cycles in the interference wave, and the more gradually does the time relation of auricular systole to ventricular systole shift. In figure 6 three types (*A*), (*B*), and (*C*) of interference waves are represented. In each type the upper

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row of squares represents auricular cycles, the lower ventricular cycles. The black squares represent auricular and ventricular systoles respectively; the white squares auricular and ventricular diastoles.

Type (*B*) represents an interference wave in which auricular and ventricular rates are approximately equal. In this instance there are seven auricular to six ventricular cycles. Auricular systole (1) is completely stoppered by ventricular systole (1) and its effects therefore annulled. Auricular systole (3) occurs at the optimum moment, just completing at the onset of ventricular systole (3). The following auricular systoles shift back on the ventricular cycles until auricular systoles (7) and (1) are again completely stoppered by ventricular systoles (6) and (1) respectively. If auricular systole is important ventricular efficiency should be at its lowest at ventricular systoles (1) and (7), and at its highest at ventricular systole (3). Such is the case.

In addition to the type (*B*) two other types of interference waves, (*A*) and (*C*), proved of value in analyzing the effects of auricular systole. In type (*A*) the ventricular rate is approximately twice the auricular rate and in type (*C*) about half the rate.

Longer interference waves, with a greater number of auricular and ventricular cycles, permit minute and progressive changes of effectiveness of auricular systole and therefore offer exceptional opportunities for studying the effects of auricular contraction on ventricular efficiency.

Since magnitude of venous pressure might influence the relative importance of auricular systole this point was studied by varying venous pressure while interference waves were occurring.

The relative filling effects of venous pressure and auricular systole might vary in the case of the thin walled right ventricle and the thick walled left ventricle. With this point in mind, simultaneous records of right and left ventricular tension were made in several experiments.

RESULTS

Since the production of auriculo-ventricular interference waves permits either annullment of the function of auricular systole or the placing of auricular systole in its most effective position in ventricular cycle, this method gives maximum value to the importance of auricular systole for ventricular efficiency. Records obtained in the course of such waves are shown in figs. 7, 15, 16 and 17. Data obtained from waves under varying conditions are given in Table I. The

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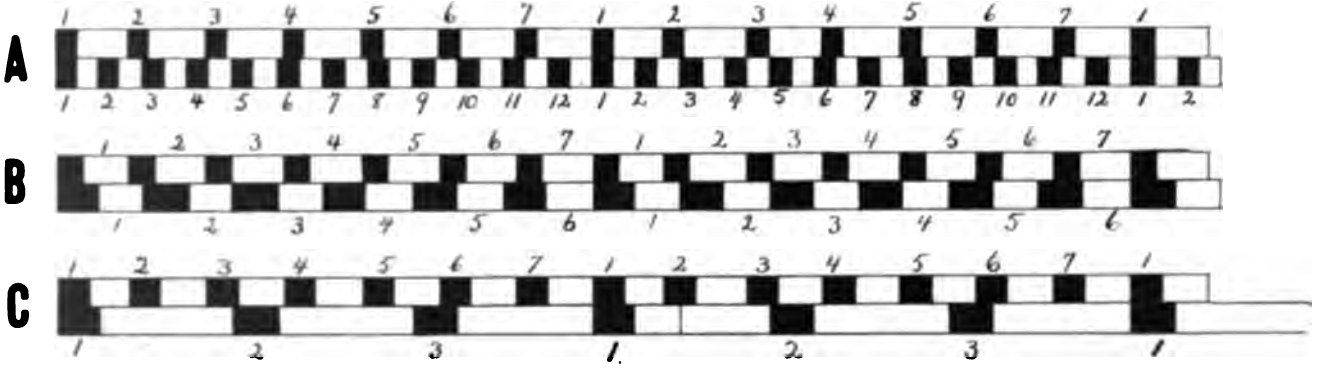


Fig. 6. Types of interference waves. *a*, Ventricular rhythm approximately twice the ventricular rhythm; *b*, auricular and ventricular rhythm approximately equal. *c*, ventricular rhythm about half the auricular rhythm. The black blocks represent auricular and ventricular systoles, the white, diastoles.

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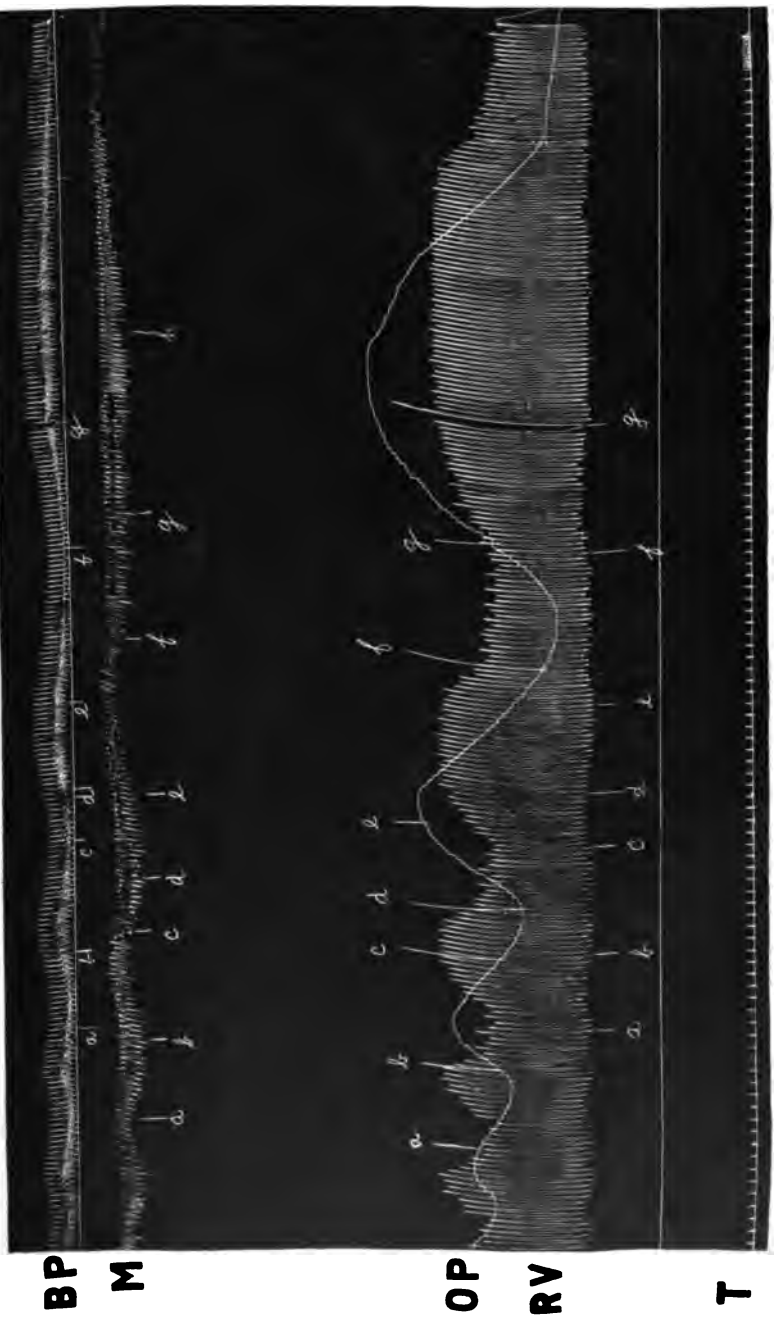


Fig. 7. Interference waves of various lengths showing fluctuation of initial and final volume length. Corresponding points are marked. *B.P.*, Arterial blood pressure recorded with the Hg. manometer; *M.*, myocardiograph tracing; *O.P.*, volume output; *R.V.*, right intraventricular pressure; *T.*, time in seconds.

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rhythmical changes, due to varying effectiveness of auricular systole, on the auricular and ventricular suspension tracings, the myocardiograph, venous pulse, volume output, intraventricular and arterial tension tracings all give evidence of the influence of auricular contraction and permit the study of auricular contraction from several points of view.

Amplitude of auricular contraction, as represented by the auricular suspension tracing, is an index to the amount of blood propelled by auricular systole. If auricular systole occurs during ventricular diastole the auricular contents is readily passed into the ventricles and auricular amplitude is large. If auricular systole is stoppered by ventricular systole the amplitude is small. This is well shown in figure 17.

TABLE I

(1) TRACING	(2) VENOUS PRESSURE IN CM. OF BLOOD	(3) VENTRICULAR RATE PER MINUTE	(4a) (4b) MINIMUM AND MAXIMUM INTRAVENTRICULAR SYSTOLIC PRESSURE IN MM. Hg.		(5a) (5b) MINIMUM AND MAXIMUM VENTRICULAR OUTPUT IN CC. PER MINUTE		(6) PER CENT INCREASE OF VENTRICULAR OUTPUT
			Min.	Max.	Min.	Max.	
44	2.7	156	115	155	280	440	57
50	3.9	114	110	140	270	430	60
16	5.3	138	115	150	370	600	62
62	5.3	298	45	142	110	400	264
38	9.0	144	120	175	650	880	35

Intra-ventricular tension tracings show fluctuations of both initial and final tension resulting from altered auricular effectiveness. See figure 16. The volume output tracings show only indirectly the effects of auricular contraction.

Table I shows the minimum left ventricular systolic tension and volume output obtaining at the trough of an interference wave—at such a time, the tension and output are maintained by venous pressure alone. It also shows the tension and output obtaining at the crest of the same wave—the tension and output maintained by venous pressure and auricular systole of maximum effectiveness. The last column in the table gives the relative importance of auricular systole, that is the percentage increase of ventricular efficiency over that maintained by the filling action of venous pressure alone.

The interpretation of these results is the primary object of this research.

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It has been suggested[9] that previous results obtained from interference waves might be adequately explained by variations of imperfection of valvular action. The possibility of disturbed valvular action accounting in part for the results obtained was pointed out at the time,[10] but was not considered important. But unless the degree of disturbed valvular action is determined, a careful analysis of other secondary factors is impossible. It, therefore, seemed advisable to study the relation of regurgitation to the results obtained.

Auricular systole immediately preceding ventricular systole may bring about better valve closure than venous pressure alone, thereby preventing regurgitation. But if the primary function of auricular systole is to insure perfect valve closure, amplitude of auricular systole presumably would have little effect on ventricular efficiency, provided auricular systole constantly precedes ventricular systole by the normal time interval. It has been shown in the case of the turtle's heart, that as the auricles undergo tonus oscillations, ventricular output varies directly as the amplitude of auricular systole. Though these experiments minimize the factor of disturbed valvular action in the mammalian experiments cited and emphasize the importance of ventricular filling, there might be some objection to applying the results directly to cardiodynamics in the mammalian heart. Valvular action was therefore further studied in the dog's heart. It seemed that the venous pulse would offer the best means for detecting the extent of regurgitation. This method was used, and in most instances a membrane manometer was employed to record the venous pressures.

The first experiments were planned with the object of determining whether auricular systole is necessary for perfect valvular action. This was done by stimulating the vagus nerve in the course of an interference wave, see figure 8. (*A*) to (*B*) represents an interference wave of the type (*C*), figure 6, in which the auricular rate is approximately twice the ventricular rate. The beginnings of auricular systoles are set off on the venous pulse tracings by the upper vertical lines, ventricular systole by the lower lines. Note the varying height of the venous waves and that the highest waves occur during interference of auricular and ventricular systole. These high positive venous waves occurring during interference may have two causes; back pressure from stoppered auricular systoles, and regurgitation at the onset of ventricular systole. By inhibiting the auricles the first cause is removed and any positive

[9] Henderson and Johnson: Heart, 1912, iv, 69.
[10] Loc. cit.

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waves must therefore have another explanation. During complete inhibition, positive waves synchronous with ventricular systole do occur, but are of too small amplitude and too slow formation to be accounted for by regurgitation from the powerful ventricular contraction. The waves probably are due to the usual negative and positive pressures obtaining during early ventricular diastole and late ventricular diastole just prior to auricular systole. Under the conditions given, auricular systole does not seem necessary to insure perfect valvular action.

The next question is: Can auricular systole abnormally placed in the ventricular cycle disturb valvular action and permit appreciable regurgitation? Such disturbance would be most apt to occur during partial interference of auricular and ventricular systoles. Such disturbance may be analyzed from two points of view; one in which ventricular systole is in progress at the onset of auricular systole and the other in which auricular systole is in progress at the onset of ven-



Fig. 8. Interference wave Type C. Fig. 6 followed by vagus stimulation. *A.*, Auricular contractions; *V.*, venous pulse. Auricular systoles are marked off on venous pulse above, ventricular systole below.

tricular systole, see figure 9. Here ventricular and auricular systoles are set off on the venous pulse obtained in the course of an interference wave of 15 auricular and 16 ventricular cycles. Ventricular systoles are enclosed in brackets above, auricular systoles in the short lines below. Examination of this record shows that in every case the positive wave follows auricular systole by a short interval of time, that the wave increases in size only when auricular and ventricular systole begin to interfere, and that the amplitude of the wave depends upon the extent of interference. Auricular systoles (12, 13, and 14) occurring during ventricular diastole, produce the smallest waves, while auricular systoles (4, 5 and 6) completely stoppered, produce the largest waves.

If ventricular systole is in progress at the onset of auricular systole the positive wave does not occur until the onset of auricular systole—indicating again that auricular systole is not essential to perfect valve closure, (see ventricular contractions 4, 5, 6 and 7). The positive waves in these instances are clearly of auricular origin.

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But when auricular systole is in progress at the onset of ventricular systole, the opportunity for regurgitation is greater. Whether regurgitation occurs under such conditions is difficult to determine definitely for a positive wave would result the moment both systoles were in progress, whether regurgitation occurred or not. Even under these adverse conditions valvular action is not appreciably disturbed, (see auricular systoles 0, 1, 2, and 3, and 15 and 16). Since ventricular systole is more powerful than auricular systole, it might be expected that any appreciable regurgitation would have a marked effect on the venous pulse. Careful examination of auricular, myocardiograph and tension tracings, however, gives occasional indication that some regurgitation may occur in one or two ventricular cycles in the course of a single interference wave. But whether such occasional regurgitation occurs or not matters little with the interpretation of results obtained from interference waves as will be seen in the following section.



Fig. 9. Venous pulse tracing taken in the course of an interference wave. Ventricular systoles are set off above, auricular systoles below.

INTERFERENCE WAVES. We can gauge the extent of regurgitation by another method of analysis, namely, by noting the intraventricular pressure obtaining in various phases of interference waves. Two types of interference waves in which auricular and ventricular rates are closely approximated can be produced; one in which auricular rate is slower, and the other faster than ventricular rate. These two types are shown in figure 10. In the upper interference wave there are 21 auricular cycles to 22 ventricular cycles. In this type auricular systole shifts forward in ventricular cycle. In the lower wave there are 22 auricular and 21 ventricular cycles. In these waves auricular sytole shifts backward in ventricular cycle.

In each case, the upper blocks represent auricular cycles, the lower ventricular cycles. The black solid blocks represent auricular and ventricular diastoles.

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Assuming that auricular systole has an enhancing effect on ventricular efficiency, and that this enhancing effect increases with the approximation of auricular systole to the normal position in ventricular cycle, and that regurgitation is not an important factor the curve of ventricular efficiency may be theoretically plotted for the two types of waves. The recorded intra-ventricular tension rather than the ventricular output is compared with the plotted curve, because the tension record is quickest to indicate changing ventricular efficiency. The shape of the efficiency curve varies in the two types, and is of value in the interpretation of results.

If auricular systoles are completely stoppered, venous pressure is the only filling force. This force is constant and, therefore, during this period, a constant level of ventricular efficiency should be maintained as shown by lines (*AB*) and *UV*), as auricular systole advances or recedes the curve should follow two different courses. This course depends on a number of factors, the discussion of which would be too lengthy for this paper. Briefly stated, it depends primarily upon the duration of auricular systole and the relative duration of ventricular systole and ventricular diastole and whether auricular systole is advancing or receding in ventricular cycle. Ventricular diastole usually is considerably longer than ventricular systole and that relation is shown in the diagram. The only difference between the two waves is the direction in which auricular systole is shifting. In the upper wave auricular systole is advancing. The effect of auricular systole (3) is annulled. Auricular systole (18) is at its optimum position. In the 15 intervening cycles auricular systole gradually shifts from a position of complete annullment to that of maximum efficiency. Ventricular efficiency for this period is therefore represented by a gradual incline (*BC*). Following auricular systole (18) auricular systole shifts from a position of maximum to one of minimum efficiency in only 4 cycles as represented by the sudden drop to the horizontal (*CA*).

The plotted curve corresponds closely with the experimental results obtained, see figure 16.

In the lower wave the reverse conditions obtain. From the constant efficiency maintained by venous pressure (*U* to *V*) auricular systole shifts in 4 cycles (1 to 5) from minimum to maximum efficiency and in 16 cycles from maximum to minimum efficiency. The curve plotted corresponds approximately with the experimental results obtained.

In connection with the question of regurgitation the sudden drop of the curve (*C* to *A*) in the upper wave might in part be accounted for

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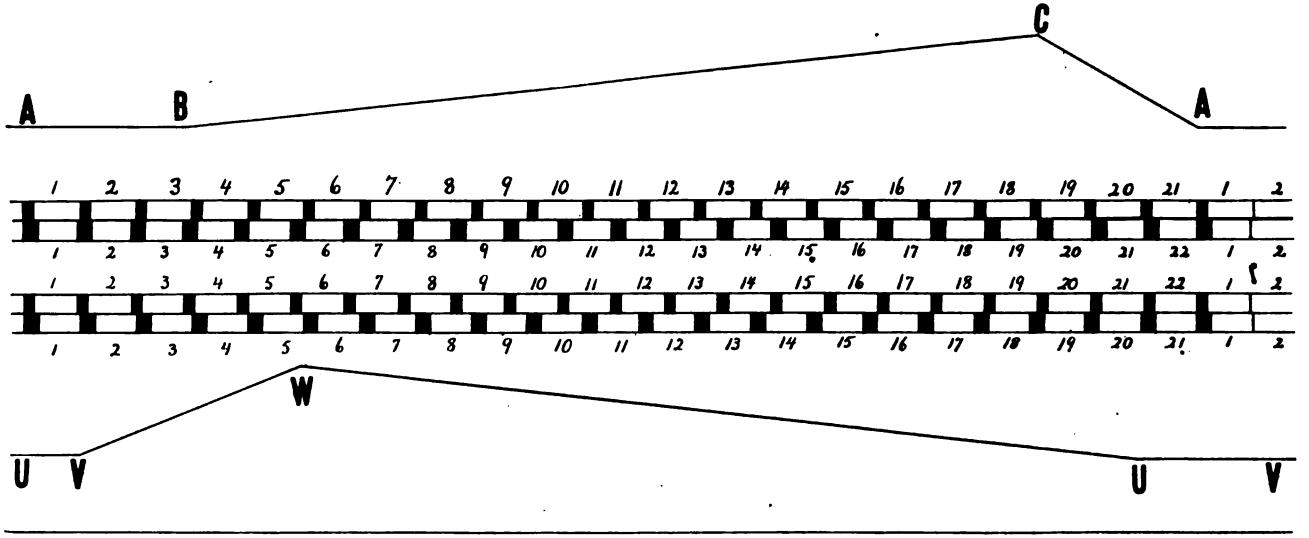


Fig. 10. Interference waves with auricular and ventricular rhythm approximately equal. Upper—21 auricular to 22 ventricular cycles. Lower—22 auricular to 21 ventricular cycles. Curves represent theoretically plotted ventricular efficiency.

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by imperfect valve action; for here auricular and ventricular systole are most dangerously interfering, i.e., ventricular systole begins while auricular systole is still in progress. The auricular valves must therefore be open. But in interference waves of type II, period (*U* to *V*) in which auricular and ventricular systole have the same relation and offer similar opportunity for regurgitation, the final intraventricular tension does not drop, but shows a rise, (*V* to *W*) as sudden as the fall (*C* to *A*) in type I. If regurgitation does occur it does not keep pace with the enhancing effects of filling due to the partially stoppered auricular systoles. In no case does the intraventricular tension during the periods in which disturbed valvular action could occur fall below that maintained by venous pressure alone (period *A* to *B*, and *U* to *V*). The oscillations in output and tension must therefore be explained by factors other than disturbed valvular action, i.e., to the enhancing effects secondary to the increased ventricular filling.

Filling Effect of Auricular Systole. If valvular action is neither dependent on, nor disturbed by auricular systole, the effects of auricular systole must be due to increased ventricular filling. Myocardiograph records show the filling effect to be considerable. (see lower tracing fig. 11 *X E* to *F*). This is a myocardiograph tracing of the ventricle with the heart in complete block. The ventricles are initiating their own rhythm of approximately one ventricular to three auricular cycles. The first sudden increase of ventricular volume is due to the filling action of venous pressure or to auricular systole, depending upon whether or not auricular contraction is in progress at the onset of ventricular relaxation. The venous pressure in this instance was 4.5 cm. of blood. The succeeding steplike increases are due in each case to the filling action of auricular systole. Note the relative importance of the two filling forces and the permanence of the filling due to auricular systole.

The filling effect is brought out still better in the myocardiograph tracing of figure 11 (*Y*) in which the auricles are inhibited by vagus stimulation, one escaped auricular contraction occurring. The only filling force is venous pressure, with the exception of one ventricular cycle, in which venous pressure and auricular systole both are effective. The venous pressure is 5 cm. of blood, the ventricular rate 72 per minute. Keeping in mind the surface-volume relation it would appear that the degree of filling due to auricular systole is even greater than that due to venous pressure. The record is of particular value in that the venous pressure is relatively high and the ventricular rate slow —offering ample opportunity for filling by venous pressure.

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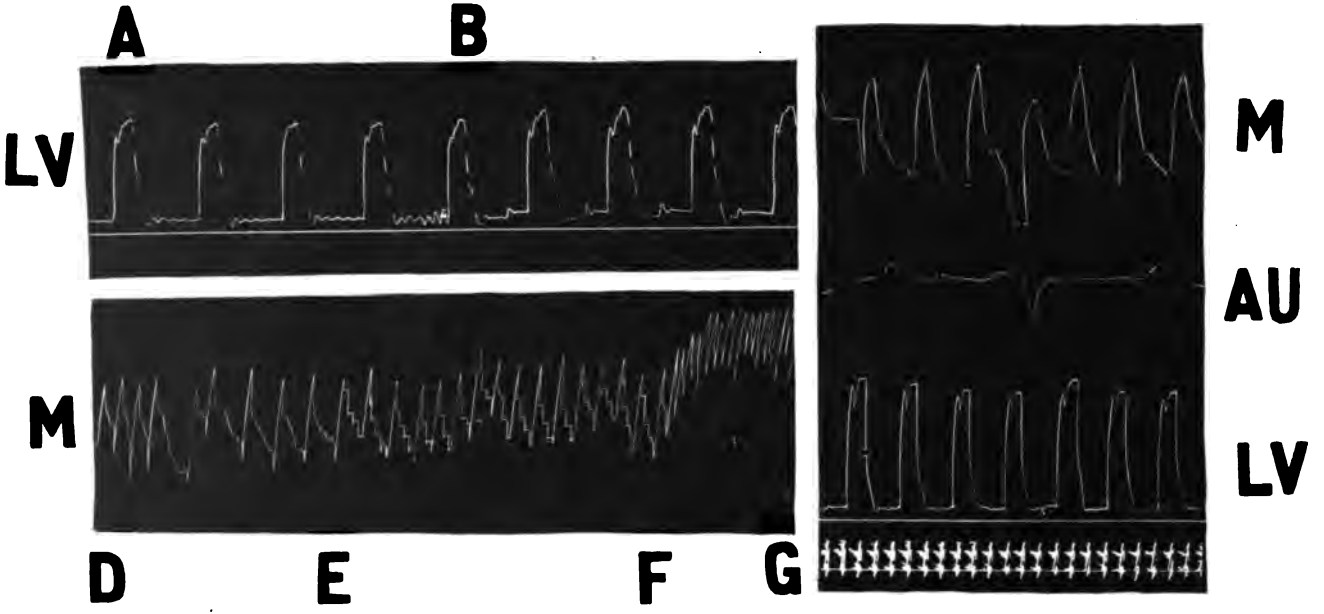


Fig. 11 (*X*). *L.V.*, Left intraventicular tension curve showing oscillations of tension due to auricular fibrillary contractions and to auricular systole. *M.*, Myocardiograph tracing showing volume length changes due to fibrillary (*D-E*) and normal auricular contraction (*E-F*). *F.G.*, ventricles stimulated at a more rapid rate.

Fig. 11 (*Y*). Escape auricular systole during vagus inhibition showing effect on ventricular volume and resulting tension curve. *M.*, Myocardiograph tracing, *AU*, auricular left contraction; *LV.*, intraventricular tension.

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Other points of interest in connection with this record are the increased duration of contraction, the increased final tension and the failure of the ventricle to reach its preceding final volume—all as a result of the escaped auricular contraction.

Though the output of this ventricular systole is considerably increased, the final volume is greater than that of the preceding ventricular systoles. With this in mind, changes in ventricular volume other than those occurring with each auricular systole may be considered in connection with interference waves (see myocardiograph tracing of figure 7). In addition to the sudden volume increase accompanying auricular systole more gradual oscillations of both initial and final volume occur rhythmically with each interference wave. At (*A*), (*C*), and (*F*) auricular systoles are stoppered and the ventricles filled by venous pressure alone. At (*B*), (*D*), and (*E*) the auricles are free to inject additional blood into the ventricles. While the effect of auricular systole is annulled, initial and final volume are at their minimum. Where auricular systole has maximum efficiency the initial and final volumes are largest. The increase of initial and final volume from (*A*) to (*B*) is due to the increasing effectiveness of auricular systole and to the increasing failure of the ventricles to empty themselves completely i.e., the auricular systole furnishes the ventricles with more blood than they can handle. Accumulation of blood in the ventricles therefore occurs. The record shows that the greater the initial volume the more poorly do the ventricles empty themselves, and vice versa, the smaller the initial volume the more completely do they empty. But even at (*C*) where the ventricles were moderately filled they fail to empty themselves completely. Such records show that ventricular volume per se is not the factor determining ventricular efficiency; but that the secondary factors accompanying the volume changes determine this primarily.

Though the volume output increases with increasing volume, the enhancing effects of the secondary factors are not sufficient to maintain the final volume obtaining during poorer ventricular filling.

ANALYSIS OF ENHANCING FACTORS ACCOMPANYING INCREASED VENTRICULAR VOLUME

The slow oscillations of volume, noted in fig. 7, resemble the volume changes described by Patterson, Piper and Starling,[11] but have a

[11] Patterson, Piper and Starling: Journ. Physiol., 1914, xlviii, 465.

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somewhat different origin. These observers find that any condition which increases the demands on the heart, whether it be increased capillary resistance or increased venous pressure, produces increased ventricular volume which in turn is accompanied by increased ventricular efficiency. In view of the relation of length of muscle fiber to strength of contraction they see in this reaction a regulative mechanism by which the blood accumulates in the heart until the length of ventricular fiber (strength of contraction) is great enough to meet the new demands. To quote from them:

> We thus find no constant connection between the diastolic tension and the succeeding contraction, though as a rule these two quantities will be altered together. But we do find a direct proportion between the diastolic volume of the heart (i.e., the length of its muscle fibers) and the energy set free in the following systole.

Further

> We see from these tracings that an invariable condition of increased contractile stress is increased initial length of muscle fiber. This may be accompanied or brought about by increase in the initial tension of the muscle fiber, but the two conditions are not invariably connected, and we shall find later other cases in which length varying without changes in tension has brought about its proper effect on the strength of contraction of muscle.

From the above it is obvious that these investigators consider length of ventricular muscle as the factor of primary importance. They do not look upon initial intraventricular tension as exerting an influence on ventricular contraction.

The contemporaneous work of Straub[12] lays stress upon another factor. Employing the same methods as Patterson, Piper and Starling, and making some of the same fundamental observations Straub arrives at entirely different conclusions. Finding ventricular diastolic tension, as well as ventricular diastolic volume to increase with increased demands on the heart, he attributes the accompanying increased ventricular efficiency to increased initial tension. In other words he considers variation of initial tension as the regulative mechanism of cardiac efficiency. He states:

> Wie die Druckkurve ausweist, bedeutet bei unseren Versuchsbedingungen die vermehrte Anfangsfüllung eine vermehrte Anfangsspannung, d. h. der diastoliche Minimaldruck ist gestiegen. In diesen Vermehrungen der Anfangsspannung

[12] Straub: Deutsch. Arch. f. klin. Med. 1914, cxv, 531.

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liegt nun der Grund, der die anpassung des Herzmuskels an die erhöhte Überlastung ermöglicht. Mit erhöhte Anfangsspannung wird nach den Zuckungsgesetzen des Skelettmuskels und des Froschherzventrikels das Druckmaximum erhöht, d. h. der Ventrikel leistet sofort erhöhte Arbeit und ist nunmehr imstande das ganze Schlagvolume gegen den vermehrten Wiederstand auszuwerfen.

From these quotations it is obvious that the views concerning the relative effects of initial length and initial tension of muscle fiber on contraction are still divided, and each factor has been considered as a means of regulating cardiac efficiency. It therefore seemed to the point to determine if possible the relative importance of these factors in contraction of cardiac muscle.

If the load of striated muscle is increased the work performed increases in a definite fashion. If the muscle is afterloaded initial length and initial tension remain constant; if not afterloaded, initial length and initial tension increase with each increase of load. The greater efficiency per given load in the second case has been attributed with equal emphasis to increased initial length and to increased initial tension. But in such experiments the two factors vary together. The difficulty of determining the relative importance of each is evident.

The properties of the auricular muscle of the turtle permit independent variation of initial length and initial tension and therefore offers an opportunity of separate analysis of these two factors, in simple experiments with conditions under easy control. In such experiments we may keep the actual as well as the filling tension constant while the length of fiber changes, and we find increased strength of contraction to accompany either increased length of fiber while initial tension remains constant or increased tension while initial length remains constant.

If these results obtained on the turtle's auricle can be applied to the mammalian heart, the work of Patterson, Piper and Starling and of Straub require broader interpretation. It would seem that under conditions in which initial intraventricular tension is low and varies but little, the factor of initial length of fiber is by far the more important of the two, but with high initial tension the factor of tension may grow in importance. But the interpretation of cardiodynamics cannot be limited to the factors of initial length and tension of the muscle fiber for another factor comes simultaneously into play. This factor is the surface-volume relation accompanying volume changes of the ventricle. The volume of a growing sphere increases more rapidly than the surface. If we consider the ventricle as roughly spherical—

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and its walls the surface—its contents the volume, the importance of this surface-volume relation is evident. It means that the greater the ventricular volume the greater the output per unit length of muscle shortening.

At least three factors are important in regulating the efficiency of the heart. When the demands on the heart are increased, ventricular volume increases until the enhancing factors of initial length of fiber, initial tension of fiber and the surface—volume relation meet the new demands.

(A) Length of Fiber. Length of fiber is the important factor determining the liberation of contractile energy. In the present experiments the final intraventricular tension was used as the gauge to strength of contraction. Since the tension developed depends on the amount of blood forced through the given resistance per unit of time, the tension developed may not depend entirely upon the amount of energy liberated but also upon the manner in which it is utilized, for instance the amount of muscle shortening and the effectiveness of the given shortening which occurs with various initial volumes. Strength of contraction or tension developed may therefore be a factor of "initial volume-length" rather than initial length alone. This term, "initial volume-length" will therefore be used to designate these factors; but where the factors of volume or length are specifically in question they alone will be mentioned.

For simplicity the factor of initial length will be arbitrarily considered alone in two given cases.

In the course of many interference waves produced in these experiments, initial right intraventricular tension varied but little as a result of varying effectiveness of right auricular systole; but the usual oscillations of initial length of ventricular fiber occurred. In such cases the final tension developed varied directly as the initial length of fiber, agreeing with the results obtained on turtle's auricular muscle (see tracing *R.V.*, fig. 7).

The same relation of final tension to initial length of fiber is also displayed by the left ventricle. During certain periods of the interference waves the left intraventricular initial tension may remain constant for a number of cycles though a decrease of initial length of fiber occurs. (See fig. 16). In such instances too, the final tension varies directly as the initial length of fiber. But, as will appear later, initial length of fiber is not the only factor determining the strength of a given contraction. The factors of initial volume, and surface-volume

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relation accompanying volume changes may influence the energy liberated in a given contraction, the effectiveness of the liberated energy and also the effectiveness of muscle shortening. It is well, therefore, to look upon the changes of final tension as a volume length effect rather than length alone.

Experiments on the auricle of the turtle showed duration of any given contractile tension as well as magnitude of final tension to be increased by increased initial length of fiber. With the conditions obtaining in the present experiments increasing initial volume-length increased duration as well as strength of contraction (see fig. 12 which shows left intraventricular tension curves only). Curves (*A*, 1 and 2) were taken from an interference wave with volume-length at its maximum and minimum respectively, that is, when auricular systole was at maximum and minimum efficiency. In this particular instance the increased duration of contraction is relatively greater than the corresponding increased magnitude of tension, a factor of no little significance. This increased duration of contraction comes out even more clearly in curves (B 1 and 2 fig. 12), in which there is only one auricular to two ventricular cycles. For ventricular cycle (*B*, 1, fig. 12) the ventricle is filled by venous pressure and auricular systole for (*B*, 2) by venous pressure alone. These cycles (*B*, 1 and 2) correspond approximately to ventricular cycles (*A*, 10 and 11, fig. 6). The difference in duration of contraction in the case of cycles (*B*, 3 and 4, fig. 12) is not so great. These cycles correspond approximately to cycles (*A*, 8 and 9, fig. 6), which explains the difference.

The practical significance of increased duration of contraction as well as strength of contraction under physiological and pathological conditions need scarcely be pointed out in this paper.

The relation of strength and duration of contraction as affected by volume length will be further elucidated in the section on surface-volume relation.

(B) Initial Tension. In this work, in no instance was initial tension the only varying factor, and no definite data concerning the effect of initial tension on ventricular contraction was obtainable. The significance of initial tension in relation to cardiodynamics must necessarily be a matter of inference. But if the results obtained on the turtle's auricle can be applied to the mammalian heart it is evident that initial tension might at times be an enhancing factor of some importance. In the course of an interference wave the initial left intraventricular tension varied from approximately zero to 20 mm. Hg, see figure 15.

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The increase of initial tension is due in part to the increased output of the right heart and in part to increasing effectiveness of left auricular systole. Whatever the cause, it means that this tension is stored in the stretched ventricular muscle during diastole as potential energy. When the muscle contracts this potential energy is liberated as dynamic energy and is effective in assisting the active contraction in expelling the blood. In addition to this mechanical factor we must bear in mind a possible enhancing effect of initial tension on the processes of muscular contraction.

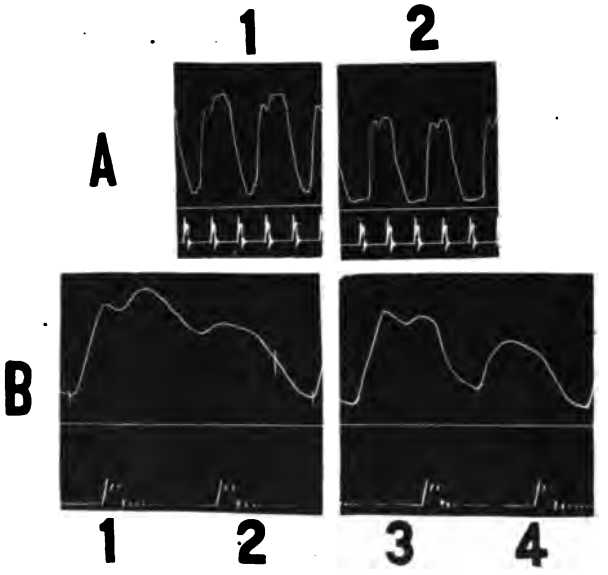


Fig. 12. Effect of auricular systole on duration and strength of ventricular contraction. *A* and *B*, left intraventricular tension curves from interference waves of type *B* and *A*, figure 6 respectively.

Patterson, Piper and Starling suggest that increased initial tension may put the muscle on a slight stretch taking up ventricular slack and thereby minimizing waste contraction. This factor is probably of more significance in the flaccid right ventricle than in the left ventricle. A further suggestion along these lines might be offered. The ventricle tends to assume a more spherical shape with increased intraventricular tension, this change occurring before the ventricle decreases in volume. If this spherical shape could be produced by increased initial tension (auricular systole or venous pressure) waste contraction would be

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decreased still more, i.e., the very first part of contraction would be effective in expelling blood, and the rate of shortening of muscle during the first part of contraction would be decreased.

It would be of interest to know the optimum initial tension for cardiac efficiency. This undoubtedly depends on a number of factors, the elasticity of the muscle, the relative economy with which the muscle sustains a high constant venous pressure and the rapid short-lived tension due to auricular systole, and the enhancing effects of various tensions on active contraction itself. It has been suggested that the sarcoplasm may under certain conditions bear the constant tension obtaining between the clonic contractions. Other suggestions are found in the literature pointing to a difference in the metabolism of sarcoplasm and of the fibrillae of muscle, one being a protein metabolism, the other a carbohydrate metabolism. It is likewise stated that resistance of smooth muscle (which possible has properties similar to sarcoplasm) to constant tension is very economic. If the above is true it is plausible that even high constant initial tension resulting from venous pressure might have an enhancing effect on muscular contraction. But the short duration of increased initial tension produced by auricular systole may be of particular value.

(C) Surface-Volume Relation. The relation of surface-volume to cardiodynamics may be considered from three points of view.

1. Influence on the effectiveness of a given contraction (muscle shortening).

2. Influence on the amount of contractile energy liberated in a given contraction.

3. Influence on the effectiveness with which the given liberated energy in utilized.

1. The influence of surface volume relation on the effectiveness of a given contraction will be considered first, for it will at the same time illustrate what is meant by that relation. The volume of a sphere varies as the cube of the radius, and the surface area of a sphere as the square of the radius. It therefore follows that the volume of a growing sphere increases more rapidly than the surface. This relation is shown in Table II for spheres of different radii. The significance of this relation, when the ventricular walls are considered as the surface and the contents as the volume, was pointed out before, and is illustrated by quantitative data collected in Table II last column:—the decrease of volume per unit decrease of surface area. The circumference-area relation of circles is similar to the surface-volume relation of spheres. It

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is therefore simpler to use circles, representing sections through the ventricles, to show diagramatically the significance of this surface-volume relation (see fig. 13).

In one instance (*A*, fig. 13) the ventricle is filled to a radius of let us say 3 cm., in the other to 10 cm. Granting in case (*A*) that the ventricular fiber (ventricular ring) shortens one-third its initial length or 6 cm. it contracts to a circle with a "volume" of 12 cm. The output is 16 cm. The same contraction of 6 cm. in case (*B*) with a radius of 10 cm. produces an output of 60 cm. But in this instance, 6 cm. is only one-tenth of the circumference. We know that a long muscle fiber contracts much more than a short muscle fiber and should the ring contract one-third its length as in case (*C*) the volume output would be 178 cm.

The application of this relation appears in the myocardiograph tracing of figure 7. At (*C*) and (*F*) where initial volume is smallest, the magnitude of contraction is small, and ventricular output is consequently

TABLE II

RADIUS	SURFACE AREA	VOLUME	VOLUME CHANGE PER UNIT CHANGE OF SURFACE
cm.	*sq. cm.*	*cc.*	
3	108	108	1.0
6	432	864	2.0
10	1200	4000	3.3
14	2352	10976	4.6
20	5024	33158	6.6

small; at (*E*) and (*G*) where initial volume is greatest, magnitude of contraction is also greatest and the output therefore is markedly increased.

An increase in volume corresponding with radii of 3 and 10 cm. would be extreme in normal hearts, but such an increase in association with the change from a normal to a pathological condition is met with and illustrates how adaptive the mechanism of dilatation is, especially when considered along with the increasing strength of contraction accompanying volume increase.

Under certain experimental conditions the volume of the ventricles in some instances probably doubled in the course of an interference wave. It is of interest to analyze the effect of surface-volume relation alone associated with such a volume change.

Taking a ventricular circle with a radius of 3 cm., a circumference of 18 cm., and a "volume" of 27 cm. the output per unit shortening

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of muscle is 1.5 cm. Doubling the "volume" produces a circle with a radius of 4.1 cm., a circumference of 26 cm., and a "volume" of 54 cm. With this larger initial volume the output per unit shortening of muscle is 2 cm. an increase of 33 per cent. But if the ring contracts in proportion to its initial length, the output is increased 60 per cent. An increase of this magnitude is commonly noted in the course of an interference wave.

Making the same assumptions in regard to ventricular relaxation as to ventricular contraction, surface-volume relation should enhance ventricular filling by venous pressure in the same way as it increases the effectiveness of a given contraction of muscle during systole.

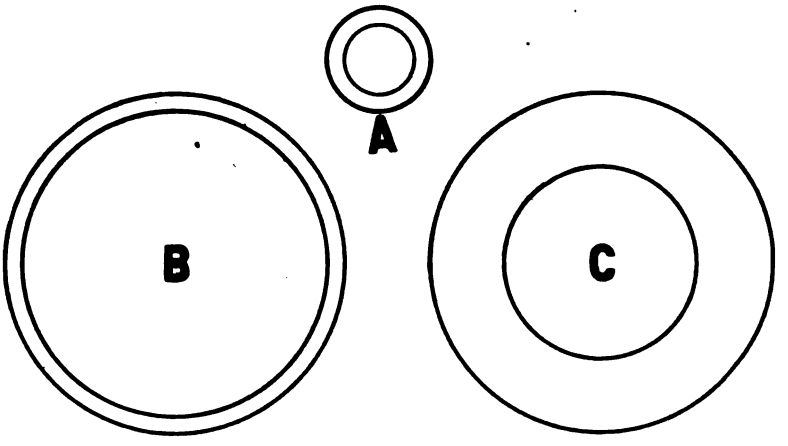


Fig. 13. Relation of surface volume to effectiveness of given ventricular muscle shortening. In *A* and *B* the contraction of the ring is equal with an output of 16 and 60 cm. repectively. In *C* contraction is proportional to that in *A*. The output is 16 and 178 cm. respectively.

Since auricular systole increases both initial and final ventricular volume it increases ventricular volume in two ways: (a) Directly—by injection of blood into the ventricles; (b) Indirectly—by supplying the ventricles with more blood than they can handle. Final ventricular volume is thereby increased and this increased final volume in itself increases fillng by venous pressure.

With the same shortening of ventricular musculature in two different initial volumes the relation of surface volume to effectiveness of contraction is obvious. This relation is important in utilizing the increased energy resulting from increased length of ventricular fiber. For example

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in some hearts when cardiac demands are increased by i creased filling initial volume alone is markedly changed, final volume may remain more nearly constant. That is the ventricle empties itself almost as well when initial volume is large as when it is small. Such instances are indicative of a rapid increase in strength of contraction as a result of increased length of fiber. If there were no means of utilizing this strength the heart would be emptied before contraction was completed. The surface-volume relation prevents this. Since the efficiency of these ventricles varied approximately as the ventricular volume, it points to the nice adjustment of strength of contraction and ventricular volume which occurs in some hearts when in good condition.

2. Influence of surface volume relation on the amount of contractile energy liberated in a given contraction. The discussion of this influence is based upon the work of Blix[13] and Hill.[14] Blix is of the opinion that the amount of contractile energy liberated in muscular contraction is a function of the exposed area of certain chemically active surfaces within the fibrillae at the time of excitation. This would make initial length of fiber the strength determining factor. Hill, however, believes that the processes at the chemically active surfaces producing the contractile energy, require an appreciable time for their completion, therefore the length of muscle during the early part of contraction as well as the initial length, determines the contractile energy liberated in any given contraction. It is in this connection that ventricular volume and surface-volume relation come into play in influencing the contractile energy liberated. The influence of initial length alone was discussed before on page 291.

As was pointed out by Patterson, Piper and Starling increased initial volume by increasing final volume would in itself insure increased length of fiber throughout contraction thereby increasing strength of contraction. This is a manifestation of surface-volume rather than of volume alone and therefore becomes of increasing importance the more poorly the ventricle empties itself. But in many instances in which the ventricular muscle is in good condition, and the demands on the heart are increased, final volume, as indicated by the myocardiograph tracing, does not increase nearly as rapidly as initial volume, i.e., the ventricle empties itself almost as well when initial volume is large as when it is small. This means that the secondary enhancing factors have been increased by increased initial volume sufficiently to handle

[13] Blix: Skand Arch. f. Physiol., 1902, xii, 52.
[14] Hill: Journ. Physiol., 1913, xlvi, 434.

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almost perfectly the blood received by the ventricles. In such instances surface-volume relation rather than the volume itself (final volume) is the factor of importance increasing both strength and duration of contraction, for the greater the ventricular volume the greater the output per unit shortening of muscle. But with a given contractile stress there is a limit to the output per unit of time and unless this stress is disproportionately increased by increased length of ventricular fiber, the tendency of the increased volume, though the muscle may contract to its minimum, will be to make the early part of contraction isometric and in that way increase the strength and duration of contraction.

3. Influence of surface volume relation on the effectiveness with which a given available energy is utilized. In the contraction of any strip of muscle we look upon the liberation of contractile energy and the resulting contractile stress as running a parallel course. That is stress is directly proportional to the liberated contractile energy. This is not the case in hollow spheroid contractile organs like the heart and was taken into account by Stephen Hales, 1733,[15] in determining the strength of ventricular contraction. In the case of a muscle strip, we have a linear pull and no changing muscle surface to consider. In the heart the muscle as a surface must support the developed tension. It is therefore possible in the course of systole for the contractile stress to increase though the contractile energy is decreasing. This would be dependent on the relative rates, with which contractile energy and the surface over which this energy is spread decrease.

The form of the tension curve produced by normal ventricular systole must therefore be dependent upon these two factors and on the amount and nature of the peripheral resistance.[16]

Assuming the ventricle to be spherical, and computing the tension developed under isometric conditions for different initial volumes, but with the liberation of equal amounts of contractile energy, we obtain data of considerable interest in relation to the importance of the internal surface obtaining with different volumes. In Tables III (*A*) and (*B*)

[15] Stephen Hales: Statical Essays, 1733.

[16] As this paper goes to press, I find that Patterson and Starling in a footnote previously overlooked (Journ. Phys., 1914, 48, 358) make a similar suggestion concerning the form of the tension curve and also point to the mechanical advantage of systole resulting from decreased surface. In the present paper, this factor is discused quantitatively as far as it can be, and is considered in connection with its varying importance with different initial volumes.

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contractile energy sufficient to produce a tension of 100 mm. Hg. when the ventricle has a radius of 3.1 cm. is used in the computation. In Table III (*A*) the intraventricular surface, the volume, and the stress

TABLE III-A

R.	VOLUME	SURFACE	STRESS
	cc.	*sq. cm.*	*mm. Hg.*
1.0	4.19	12.6	952.5
1.3	9.21	20.9	570.1
1.6	17.13	32.2	372.7
1.9	28.70	45.3	264.4
2.2	44.62	60.8	197.3
2.5	65.36	78.5	152.9
2.8	91.97	98.5	121.5
3.1	124.86	120.0	100.0
3.4	164.66	145.1	83.0
3.7	212.22	171.9	69.8
4.0	268.16	200.9	59.9
4.3	333.10	232.2	51.7
4.6	407.91	265.0	45.2
4.9	492.95	301.5	39.8

TABLE III-B

CONTRACTION FROM $R^1 - R^2$	VOLUME OUTPUT	PER CENT INCREASE OF TENSION. END OF SYSTOLE (R^2) COMPARED WITH BEGINNING OF SYSTOLE (R^1)	PER CENT INCREASE OF TENSION, IF OUTPUT WERE 50 CC. IN EACH CASE
R^1 R^2	*cc.*		
4.9 — 4.0	224.8	50.5	11.2
4.6 — 3.7	195.7	54.4	13.9
4.3 — 3.4	168.4	60.5	18.0
4.0 — 3.1	142.3	66.9	23.5
3.7 — 2.8	120.3	74.0	30.8
3.4 — 2.5	99.2	84.2	42.4
3.1 — 2.2	80.2	97.3	60.7
2.8 — 1.9	63.2	118.6	93.8
2.5 — 1.6	48.3	143.8	148.8
2.2 — 1.3	35.4	189.0	267.0
1.9 — 1.0	24.5	298.0	607.9

developed in ventricles with different radii are given. This relation of stress to the radius, that is, the effectiveness of a given energy to develop tension with varying size of the ventricle is plotted in figure 14, *X*. This figure shows the varying importance of the surface-volume re-

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lation with different initial volumes—for the efficiency of the contractile energy increases more rapidly per unit of muscle shortening, the smaller the initial volume, a factor which would point to an optimum initial volume for maximum utilization of contractile energy. The significance of this point is brought out in Table III (*B*). Column (1) represents ventricular contractions from initial radius (R') to final radius (R^2). These contractions are all of equal magnitude. Column (2) represents the output resulting from these contractions; column (3)

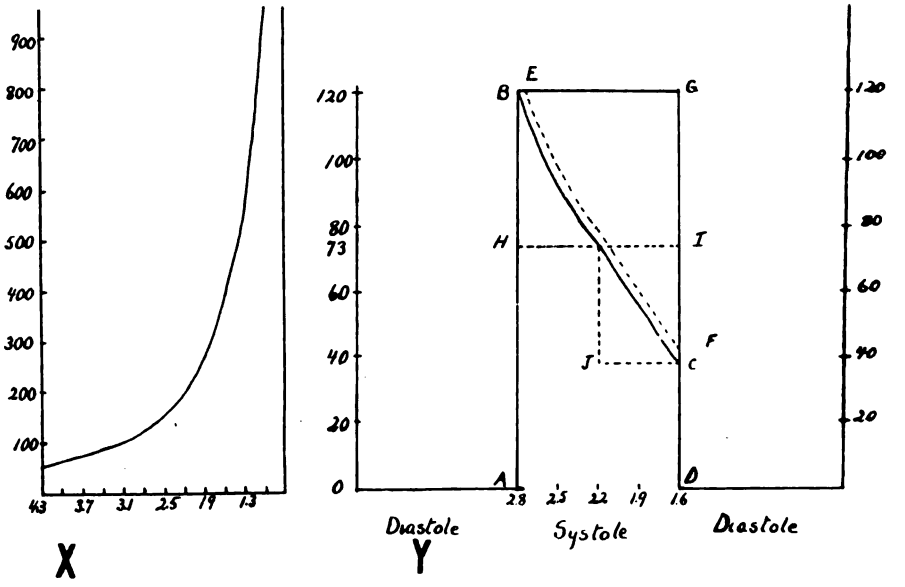


Fig. 14. *X*, Curve of contractile stress for a constant amount of contractile energy liberated in spheres with different radii. Ordinates—stress in mm. Hg. Abscissae—radius of spheres. *Y*, Hypothetical tension curve with a given curve of liberated contractile energy and a parallel curve of decreasing intraventricular surface. (For further explanation see text.)

represents the resulting increased efficiency of the contractile energy, i.e., the tension developed with the final radius as compared with that developed with the initial radius. Note that the effectiveness with which the liberated energy is utilized increases rapidly as the initial volume decreases, even despite the decreasing output occurring with the equal muscle shortening. But assuming a constant and approximately normal systolic discharge of 50 cc. the increasing effectiveness of

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the contractile energy is very much greater (see Column 4). With such a discharge the increased effectiveness of the energy is only 11 per cent when the initial radius is 4.9 cm. and 607 per cent when the initial radius is 1.9 cm.

Normal ventricular volume relations in the ventricular cycle are approximated in the contraction from a radius of 2.5 cm. to 1.6 cm. The output is 48 cc. and the residual volume 17 cc. In such an instance the resulting decreasing surface would produce increased efficiency of the energy for development of tension of approximately 148 per cent.

But the available contractile energy in muscular contraction does not remain constant throughout systole as assumed in these hypothetical cases. Consequently the ability of the ventricles to develop tension will depend largely upon the nature of the processes of contraction, particularly upon the rate at which the contractile energy is liberated or stored as potential energy and the rate at which this energy is dissipated.

Upon muscular excitation, according to Hill[17] certain processes occur at the chemically active surfaces which produces a change in the elasticity of the muscle thereby storing potential energy which is at the disposal of the muscle to perform work or develop heat. According to Hill, this new state of elasticity is of short duration. If the muscle is given an opportunity to contract during that period, work is performed, if not, the potential energy is dissipated as heat. At excitation therefore potential energy is stored just as when muscle is actively stretched. Applying this conception to decreasing intraventricular surface during ventricular systole we see a compensating mechanism between decreasing intraventricular surface and decreasing available energy. Assuming the muscle to be elastic, comparable to rubber tissue, in the so called stretched condition at the onset of contraction, the potential or available contractile energy is at its maximum. As the muscle shortens the contractile energy diminishes but counteracting this, is the decreasing surface which increases the efficiency of the remaining contractile energy. If in the course of muscle shortening the contractile energy is dissipated at the same rate as the intraventricular surface decreases, the tension curve would have a horizontal plateau. Such conditions are plotted in figure 14 (*Y*). Curve (*ABCD*) represents the liberation and dissipation of contractile energy in the course of systole, (*AB*) the sudden liberation, (*BC*) the dissipation during shortening and

[17] Loc cit.

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(*CD*) the sudden cessation of the new elastic state of the muscle. (*EF*) represents the decreasing intraventricular surface, (*ABGD*) the resulting tension curve.

The significance of this relation is apparent. It works toward complete utilization of liberated energy and minimizes waste residual contraction. For example if the peripheral resistance during systole is represented by (*HI*) the entire systole is effective in expelling blood. If the decreasing surface did not accompany decreasing available energy there would be a waste residual contraction (*JC*) amounting to fifty per cent of the entire contraction. It seems that this compensating mechanism between decreasing intraventricular surface and decreasing strength of contraction must play an important part in cardiodynamics, whether Hill's conception of muscular contraction holds or not. A breaking down of this mechanism would result in residual waste contraction. For instance, should the contractile energy be dissipated faster than the surface decreases, a tension lower than the peripheral resistance might result. Under such conditions there would occur prolonged contraction at the end of systole with the development of considerable tension below peripheral resistance but with no output. Such maintenance of tension without output does occur as shown by Patterson, Piper and Starling and others, and it would seem that its explaination consists in the failure of the surface effect to compensate the decreasing energy.

In regard to the question of the most efficient relation of available contractile energy to the supporting surface it is of interest to analyze the nature of ventricular contraction. Ventricular systole meets with two resistances: capillary resistance and stretching of the arterial walls. Capillary resistance remains constant. Since the resistance to stretching increases with filling, the ventricular contraction resembles auxotonic contractions in which resistance to shortening of the muscle increases as contraction proceeds. The major work of the heart is the storage of potential energy in the arterial walls. In this connection it would seem that the decreasing surface tending to increase or maintain the strength of contraction as systole proceeds and resistance increases is particularly valuable.

As a principle in cardiodynamics the decreasing surface must be important; but as a factor in the "adaptive volume reaction," that is the increased initial volume occurring whenever cardiac demands are increased, the relation is not so clear. From Table III (*B*), however, it would appear that the disadvantage to the ventricle as far as economic

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utilization of energy is concerned is considerably increased with increase of initial volume; for with the same given output the increased efficiency of a given constant energy, is approximately 607 per cent with a radius of 1.9 cm. and only 11 per cent with a radius of 4.9 cm. This question however needs amplification. The effect of initial volume on utilization of energy depends upon our conception of the nature of muscular contraction. If Hill's explanation is accepted, the disadvantage of increased volume is not as great as appears in the Table III (*B*). For the available contractile energy during any period of contraction would depend largely upon the amount of muscle shortening. Since the shortening with large initial volume is less per given output, the compensating mechanism of decreasing surface is not so essential. But the available contractile energy in muscle is not merely a matter of shortening; it is also a factor of time, for the contractile processes last a short time only. They develop and disappear in a definite fashion. In isometric contractions, tension rises suddenly, is maintained for a short time at a varying level, and then falls, but at a much slower rate than the rise. This fall of tension in isometric contraction is a factor of time, for the muscle does not shorten and this decreasing available energy as affected by time would in all cases be more economically utilized when initial volume is small than when initial volume is large.

The relation of surface to tension leads indirectly to the question of the limit of the adaptive volume reaction in response to increased cardiac demands. Most of the factors involved have been analyzed. It is seen that these factors vary in relative importance and in different proportions under varying conditions; that they are inseparably interrelated and affect each other in different ways:

Increased initial length and increased initial tension increase the strength of contraction. Increased initial volume by maintaining a greater length of fiber throughout contraction likewise increases the contractile energy liberated. The manner in which surface-volume relation increases the duration of contraction and the energy liberated, and the effectiveness of a given shortening of muscle have been discussed. Most of the factors mentioned are the result of increased ventricular filling and tend to increase strength of contraction. But accompanying increase in strength of contraction there are certain antagonising factors which eventually lead to the breaking down of the adaptive volume reaction. From Table III (*A*) it would appear that chief among these factors is the increasing intraventricular surface

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accompanying volume increase.[18] In addition the relatively smaller decrease of surface per unit output with larger initial volumes may lead to less economic use of the expended energy than occurs with smaller initial volume and in turn lead to collapse. Whether the high constant initial tension obtaining in extreme cases exerts a deleterious or enhancing effect on cardiac efficiency is difficult to state.

In some hearts all the factors mentioned work so smoothly that the two primary factors of energy and effectiveness of the given energy are well coordinated. With the ventricular muscle in good condition increase of initial volume from increased demands may lead to a much smaller increase of final volume, i.e., the efficiency of the ventricles varies approximately as their volume giving the impression that volume per se is the factor determining ventricular output, when in fact, the factors secondary to increased initial volume are working so perfectly that they are not obvious. But if these hearts are exhausted or the demands increased, the working of the secondary factors becomes more apparent. If the increased strain on the heart becomes too great the adaptive volume reaction suddenly breaks down. The reason is obvious. The effect of increasing strength of contraction is increasingly counteracted by the factors mentioned. When these two antagonising factors equalize each other the heart is in a precarious condition. Any additional strain would produce collapse.

Another point of interest is a detail in the method by which the ventricles meet increased demands. The demands on the ventricles may be increased in two ways: (1) by increasing the peripheral resistance; or (2) by increasing ventricular filling. The first method was not studied in this group of experiments. But Patterson, Piper and Starling, and Markwalder and Starling[19] find that when the peripheral resistance is raised, ventricular volume increases until the output is equal to that obtaining at the original resistance. Since heart rate is constant it follows that with the larger initial volume the actual contraction of the ventricular fibers is less though the energy liberated in each contraction is increased. But if the demands on the heart are increased by increasing ventricular filling, e.g. progressively increasing the effectiveness of auricular systole in an interference wave, the initial volume increases, but in this case the volume output and magnitude of contraction likewise increase, see figure 7.

[18] Patterson and Starling: Journ. Physiol., 1914, xlviii, 357.
[19] Markwalder and Starling: Journ. Physiol., 1914, xlviii, 348.

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RELATIVE IMPORTANCE OF VENOUS PRESSURE AND AURICULAR SYSTOLE

Venous pressure and auricular systole are the two important forces producing ventricular filling. Since interference waves give the output maintained by venous pressure alone and by combined venous pressure and auricular systole, they offer an opportunity for determining the relative importance of each with varying magnitudes of venous pressure. Table I gives some of the result obtained. Henderson states that auricular systole is important only when very low venous pressures exist. According to him, auricular systole has no filling effect when the venous pressure is above the "critical" pressure of 5 cm. of blood.[20] But it will be noticed that even with a venous pressure of 9 cm. of blood auricular systole increased ventricular output 35 per cent over that maintained by venous pressure alone (see fig. 17); and in other experiments, in which output was not measured, marked oscillations of blood pressure occurred in the course of interference waves though venous pressure was as high as 15 cm. of blood. It might be expected however, that the importance of auricular systole would fall off markedly with increasing venous pressure. But this does not necessarily follow, for auricular systole is an additional force to venous filling and any additional force should add its filling effect, thereby increasing ventricular efficiency. In addition the effectiveness of auricular contraction is increased by increased venous pressure in every respect just as is ventricular contraction increased by increased ventricular filling. As long as increased ventricular volume increases ventricular efficiency,—auricular systole should be effective in increasing this efficiency regardless of the venous pressure obtaining. No definite statement can be made with regard to the relative importance of venous pressure and auricular systole. It depends on many variable factors,—strength of auricular systole, duration of ventricular diastole, rate of ventricular relaxation, and resistance which ventricular muscle offers to stretching. This is brought out in tracings (16) and (62) Table I taken from the same animal with same venous pressure. In one case ventricular rate is 138 in the other 298 per minute. In the latter auricular systole is relatively more important than in the former. This probably is due to the shortened period of diastole, and to the fact that the venous pressure was not sufficient to produce ventricular stretching, while auricular systole was.

[20] Henderson and Barringer: This journal, 1909, xxxi, 352.

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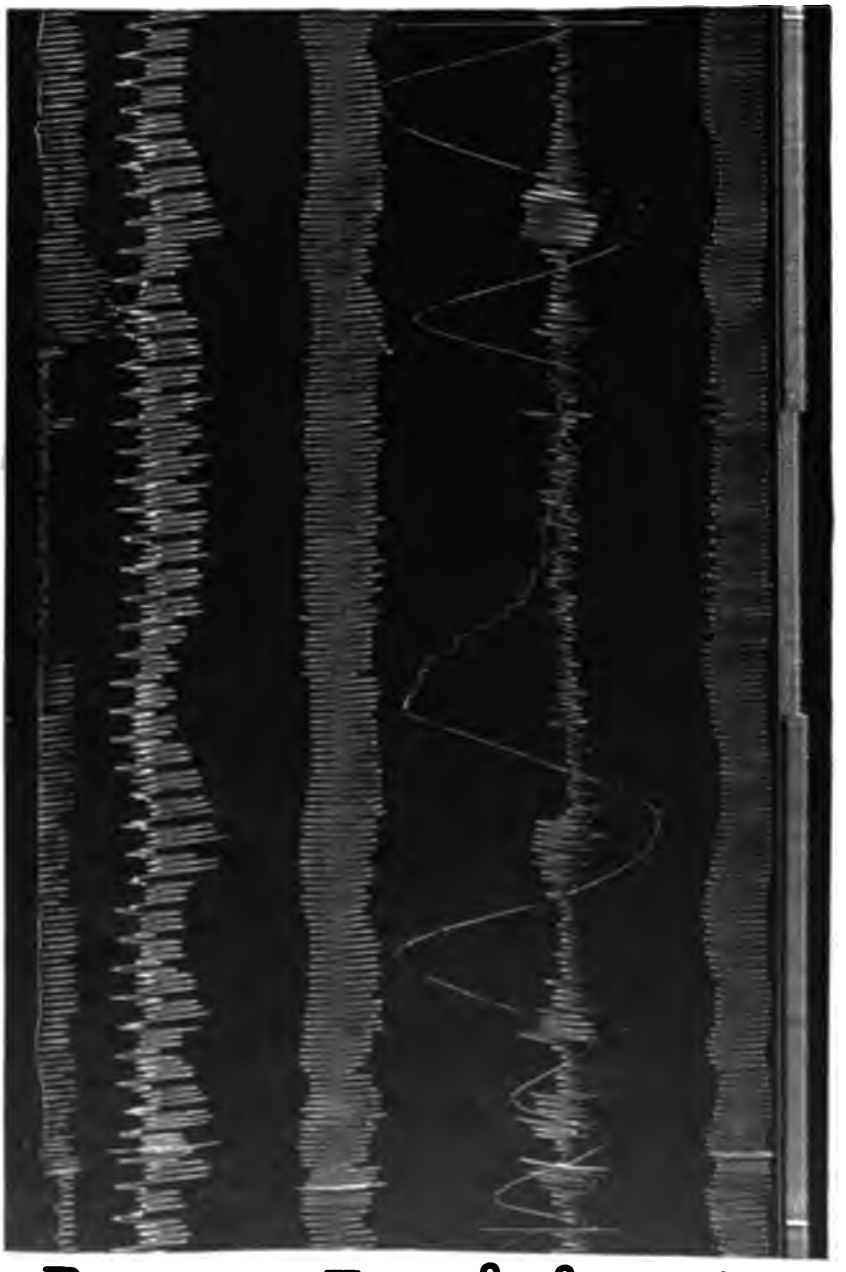


Fig. 15. Effect of faradic stimulation of auricles at the crest of an interference wave. *AU.*, auricles; *V.*, ventricle; *M.*, myocardiograph; *O.P.*, left ventricular output; *V.P.*, venous pulse; *L.V.*, left intraventricular pressure; *T.*, time in seconds.

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INFLUENCE OF SO-CALLED AURICULAR FIBRILLARY CONTRACTIONS ON VENTRICULAR EFFICIENCY

The so-called fibrillary contractions of the auricles produced by faradic stimulation are usually considered as having little filling effect upon the ventricles. This attitude may largely be accounted for by the association of these contractions with fibrillary contractions as seen in the ventricles which as is well known have practically no efficiency.

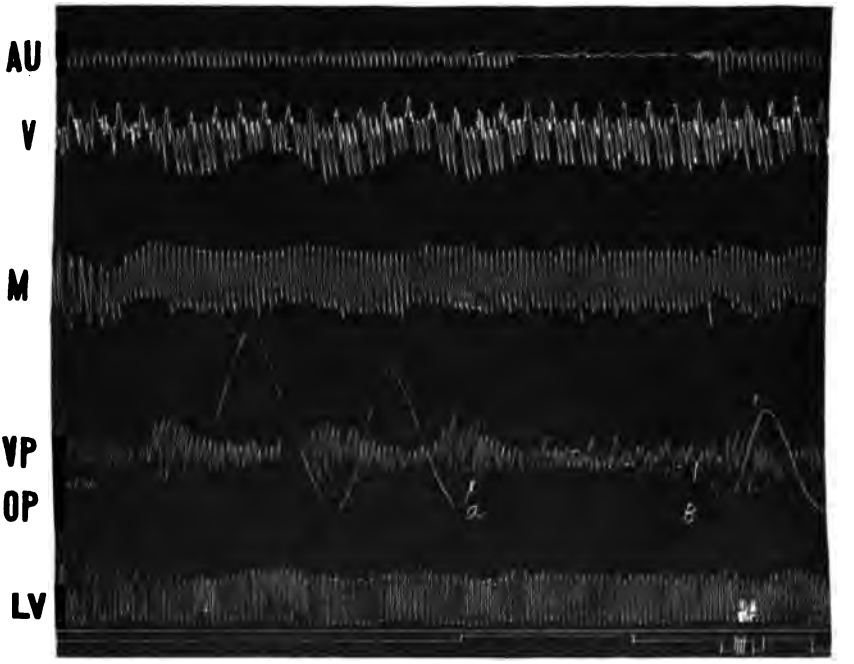


Fig. 16. Effect of faradic stimulation of the auricles at the trough of an interference wave. Stimulation at *A*; recovery at *B*.

It is pertinent to discuss briefly the nature of auricular contractions as obtained by faradic stimulation of the auricles.

Two types of auricular contraction resulting from such stimulation, as studied by Robinson[21] are well known—fibrillary contractions as seen in the ventricles, and small rapid contractions involving the major part of the auricular muscle, occurring at the rate of about 500

[21] Robinson: Journ. Exp. Med., 1913, xvii, 429.

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per minute. In the present experiments this "*auricular tachycardia*" was also in evidence, but the extent of the accompanying *fibrillary contractions* was not determined by the method employed. The rapid contractions seemed coordinated, and of a constant rate, approximately 650 per minute. Their amplitude varied considerably, but in all cases was smaller than the amplitude of normal auricular systole.

The influence of these contractions is easily determined by stimu-

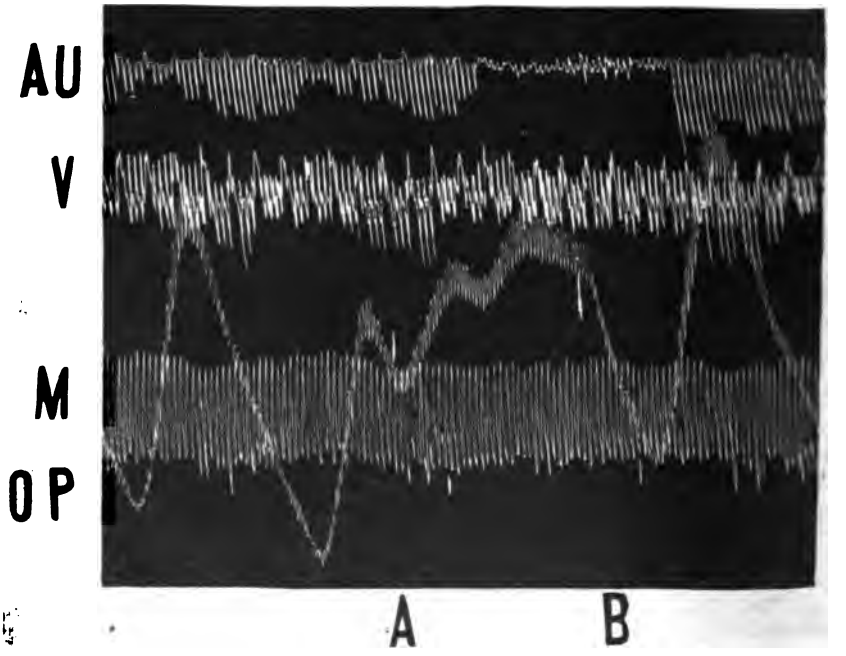


Fig. 17. A record showing very efficient fibrillary contractions of the auricles. Period of fibrillation *A-B*. *AU.*, Auricular contraction; *V.*, ventricular contraction; *M.*, myocardiograph; *O.P.*, volume output.

lating the auricules at the crest and at the trough of an interference wave see figure 15 and 16. In figure 15 the auricles are stimulated at the crest, when auricular systole is at its maximum efficiency. The output falls, but does not reach the minimum level maintained by venous pressure alone. In figure 16 the auricles are stimulated at the trough of the wave. In this case the output increases considerably over that maintained by venous pressure. In all cases these contractions had a very appreciable beneficial effect on ventricular efficiency,

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and in some instances maintained an output very little below that maintained by combined venous pressure and auricular systole, properly placed. Such an instance is shown in figure 17. The period of rapid contraction is included between points (*A*) and (*B*). During this period there are small oscillations in the volume output tracing. These probably are due to rhythmical interference of the auricular and ventricular contractions.

The explanation of the effectiveness of these rapid contractions is suggested in figure 11. In this figure the lower is a ventricular myocardiograph tracing, the upper a left intraventricular tension tracing. (*A-B*) represents a period of rapid contractions (*B-C*) recovery to

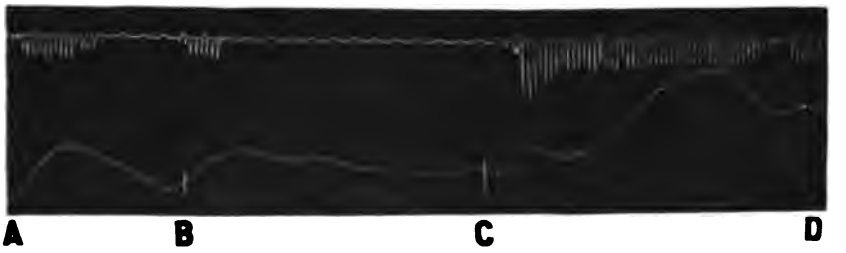


Fig. 18. A record showing that the relative efficiency of fibrillary contractions of the auricles depend on the magnitude of normal auricular systole. *A-B*, Interference wave with normal auriuclar systole. *B-C*, Period of auricular fibrillation; *C-D*, Interference waves in which magnitude of auricular systole is increased. Upper tracing—Auricular contraction. Lower tracing—Volume output.

normal contraction. The upper tracing shows the development of intraventricular tension with each auricular contraction to be considerable. The effect of these contractions on ventricular volume appears in the myocardiograph tracing. Each steplike increase of volume-length in period (*D* to *E*) is the result of an individual auricular contraction. Compared with the period following (*E-F*) where auricular contractions are normal, the filling is quite as effective.

The relative effectiveness of the so-called fibrillary contractions as compared with the normal auricular contractions depends on two factors—the magnitude of the normal contraction and of the fibrillary

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contractions, both of which can vary. Figure 18 illustrates this point. (*A* to *B*) represents the usual auriculo-ventricular interference wave, with the auricles contracting normally; (*B* to *C*) the auricles are fibrillating; at (*C*) the auricles recover and are contracting with increased intensity, the usual result of previous fibrillation. This record illustrates well the importance of magnitude of auricular systole as well as the importance of proper time relation.

EFFECT OF VAGUS STIMULATION ON VENTRICULAR EFFICIENCY

Stimulation of the vagus nerve with the heart in block effects the auricles primarily. By annullment of auricular systole in the course of an interference wave we might expect ventricular efficiency to fall approximately to that represented by the trough of the interference wave—the efficiency maintained by venous pressure alone. This obtains in figure 19. On stimulating the vagus at the crest of a wave,

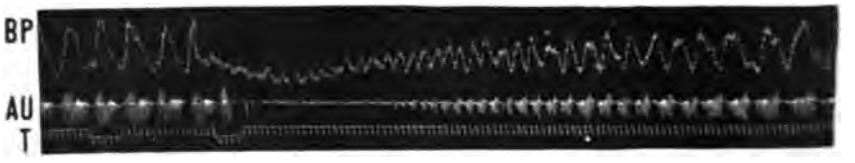


Fig. 19. Effect of stimulation of the vagus nerve in the course of interference waves. *B.P.*, Arterial blood pressure recorded with the Hg. manometer; *AU.*, Auricular contraction; *T.*, Time in seconds.

the blood pressure falls rapidly to the level of the trough. But this sudden drop is followed by a small and gradual drop, the interpretation of which is more difficult. It may be explained by the observation of Erlanger[22] who finds that vagus stimulation with the heart in block has a small, but retarding effect upon ventricular rate. This same effect was noted in the present experiments and occurred apparently to the same degree whether the bundle was completely crushed or only temporarily pierced by the hook of the clamp. Erlanger noted further that the latent period of the retarding effect was longer in case of the ventricles than in case of the auricles. This could account for the delay of the second fall of pressure seen in the present experiments. Since ventricular rate was maintained constant in figure 19 by direct stimulation, the fall of pressure, if a direct vagus effect on the ventricles occurred, would be accounted for by effects other than chronotropic.

[22] Erlanger: Arch. f. d. ges. Physiol., 1909, cxxvii,

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Other factors may likewise contribute to this fall of pressure—a more complete emptying of the ventricles progressively decreasing initial volume and thereby decreasing ventricular efficiency, or poorer ventricular nourishment resulting from the long maintained low pressure.

Figure 19 illustrates again the significance of magnitude of auricular systole. As auricular systole slowly recovers from vagus stimulation the magnitude of the blood pressure waves progressively increases.

SUMMARY

An attempt was made to determine the relation of ventricular efficiency to ventricular filling and to analyze and correlate the various effects of auricular contraction on cardiodynamics.

This was done with a modified heart-lung preparation of the dog, with the heart in block, by varying the magnitude of venous pressure and the effectiveness of auricular systole.

With the methods employed, rate of auricular and ventricular contraction, nature of auricular contraction, time relation of auricular to ventricular systole, venous pressure, and capillary resistance were under control.

By recording auricular and ventricular contractions, variation of length of ventricular fiber, which indirectly gives ventricular volume changes, right and left intraventricular tension, venous pulse, and left ventricular output, the effects of auricular systole were analyzed.

In connection with the methods used, a piston-myocardiograph, a differential volume recorder, a trocar-cannula and a pneumatic blood pump are described.

The importance of auricular systole was determined by the use of auriculo-ventricular interference waves.

Under the conditions of these experiments auricular systole increased ventricular output about fifty per cent over that maintained by venous pressure alone.

No definite statement can be made concerning the relative effect of auricular systole with different venous pressures. This depends largely upon duration of ventricular diastole, resistance of ventricular muscle to stretching, etc.

Reasons are given why the variations of ventricular efficiency in the course of an interference wave cannot be ascribed to disturbed valvular action.

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Since even a moderately filled ventricle does not empty itself completely, ventricular volume per se cannot be the factor determining ventricular efficiency, but rather factors secondary to volume change.

Auricular systole means: (1) increased length of ventricular fiber, (2) increased initial intraventricular tension, and (3) altered surface-volume relation, all of which enhance ventricular efficiency.

1. Increase in length of fiber increases the strength of contraction by increasing the liberation of contractile energy. Duration as well as strength of contraction is markedly increased.

2 *a*. Increased initial tension increases the strength of contraction through the potential energy thereby stored in the ventricular walls. This potential energy is liberated as dynamic energy during systole and in turn helps to expel the blood.

b. This increased initial tension may also have a specific enhancing effect upon the processes of muscular contraction.

c. By minimizing initial waste contraction, and by tending to slow the early part of ventricular contraction, increased initial tension increases ventricular efficiency.

3. The changing surface-volume relation influences cardiodynamics in a number of ways:

a. It influences the effectiveness of a given muscle shortening by virtue of the fact that the volume of a sphere increases more rapidly than the surface; which means that the greater the ventricular volume the greater the output per unit shortening of muscle.

b. It increases the amount of liberated contractile energy by retarding muscle shortening during the early part of contraction thereby, according to Hill, increasing the amount of liberated contractile energy.

c. It increases the efficiency of the available contractile energy in ventricular systole by virtue of the fact that the intraventricular surface over which the available energy is spread, decreases as systole progresses.

The manner in which the latter factor might play an important part in cardiodynamics is discussed.

Since the relative importance of the secondary factors vary in different proportions under varying conditions, since they affect each other in different ways, and are inseparably interrelated, it is impossible to allot to each its relative value.

The increase in ventricular volume noted on increasing cardiac demands is an adaptive reaction in which initial length of fiber, initial intraventricular tension, and surface-volume relation play a part.

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The reaction suddenly breaks down when the counteracting factors become greater than the accompanying increasing strength of contraction.

Auricular fibrillation increases ventricular efficiency in a manner similar to auricular systole. Although the effects are not as marked, at times the so-called auricular fibrillary contractions are nearly as efficient as normal auricular contractions.

Stimulation of the vagus nerve, in the course of interference waves by annulling auricular systole results in ventricular efficiency approximately equal to that maintained by venous pressure alone.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XXXIII. The Secretion of Gastric Juice in Cases of Gastric and Duodenal Ulcers

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No one has reported any direct and definitely controlled experiments with pure gastric juice either in clinical or in experimentally produced ulcers. It is commonly stated that in an ulcer of the stomach or duodenum the gastric glands undergo changes which result in any one of the following conditions: (1) Hypersecretion; (2) Hyperacidity; (3) Hyposecretion; (4) Hypoacidity.

Since this leaves the question of hyperacidity and hypersecretion in gastric ulcers unsettled, the present work was undertaken at the suggestion of Dr. Carlson, in the hope of securing data better controlled than is possible in man.

Pavlov (1) has reported one instance of spontaneous gastric ulcer in a dog with a Pavlov pouch. The ulcer lodged in the pouch, and he stated there resulted a hypersecretion but no hyperacidity. Stanley (2) concludes from eighteen cases of gastric ulcer that there is an actual increase in the acidity of the gastric contents but no hypersecretion. The maximum total acidity which he obtained was 0.40 per cent and the maximum free acidity was 0.35 per cent. The average total acidity was 0.31 per cent and the average free acidity was 0.32 per cent. Patterson (3) gives the following four analyses of gastric contents, which are typical of a number of cases: In duodenal ulcers distal to the pylorus, the total acidity is 0.32 per cent and the free is 0.02 per cent. In duodenal ulcers close to the pylorus, the total acidity is 0.3 per cent and the free 0.016 per cent. In gastric ulcers close to the pylorus the total acidity is 0.3 per cent, the free is 0.016 per cent and the protein HCl is 0.082 per cent. In gastric ulcers located in the middle of the stomach the total acidity was 0.321 per cent, the free is 0.01 per cent, and the protein HCl is 0.0824 per cent. He states, however,

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that the analyses of the gastric contents alone, in the absence of the other symptoms of ulcer, has no diagnostic value. Rehfuss and Hawk (4) found certain deviations in the concentrations of the acidity and in the secretion curve in various pathological conditions. They make the statement that in most of the cases there is present a condition of hypersecretion but give few figures to substantiate such a conclusion. Christiansen (5), Michaelis and Davidson (6) determined the acidity of the gastric contents in cases of dyspepsia, gastric cancer, gastric ulcer and many other pathological cases. They found considerable variation in the acidity but in no instance was the acidity above 0.43 per cent. Neilson (7) makes the statement that hyperacidity is frequently encountered in cases of gall stones, floating kidney, hyperthyroidism, chronic appendicitis and ulcer of the stomach. Wolpe's (8) report is in direct contradiction to the statement of Neilson. He found achylia constant in all cases of pronounced types of exophthalmic goiter. Even when one of the classic triad of symptoms was lacking, the secretion of HCl did not seem to be modified.

The fact that clinicians are usually dealing with a mixture of juices (bile, pancreatic, salivary, and gastric) may account for the many contradictory results obtained in normal and pathological conditions. In no instance where hyperacidity is reported does the concentration of acid exceed 0.55 per cent. Boldyreff (9) in a recent review of the literature has shown that where almost pure normal human gastric juice was obtainable, the acidity expressed in per cent of HCl was from 0.35 per cent to 0.48 per cent. Carlson (10) and others have shown that normally in man the appetite juice has an average acidity of 0.45 per cent and may reach a total acidity of 0.55 per cent without showing any of the so-called symptoms of hyperacidity. Pavlov (11), Foster and Lambert (12), and many others have reported similar high concentrations in dogs.

The work done on the experimental production of gastric ulcers up to 1906 has been very thoroughly reviewed by Turck (13). Many workers have since been engaged in the production of gastric ulcers by other means. Friedmann and Hamburger (14, 15) have produced chronic and acute ulcers in dogs by tying a silk ligature loosely around the pylorus, thus producing partial pyloric stenosis, after which they injected 1 cc. of 5 per cent solution of silver nitrate directly into the mucosa. No cultures were made of the ulcers thus produced so that one is left in doubt as to whether the chronicity of such an ulcer is actually due to the impairment of motility, or to an infection of the area destroyed by silver nitrate. Before and after the production of the gastric

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ulcer, test meals were given to the dogs, and after 50 minutes the stomach contents were removed and analyzed. In their tables they give figures showing a "hypersecretion" in some cases and a "hyperacidity" with a maximum total acidity of 0.3 per cent. They did not consider the factor of continuous secretion, so that what they called "hypersecretion," might be due to an excess of continuous secretion or a prolonged secretion as a result of stasis of food. Relatively few experiments were made on each dog and the data they publish might easily come within the normal variations in the volume and acidity in the gastric contents in any one dog. Their work meets with the same criticism as the work done on human beings, namely, in the analysis of the gastric contents, they do not deal with pure gastric juice.

Rosenow (16) produced chronic and acute gastric ulcers in dogs by intravenous injections of certain strains of streptococci. He showed that pure cultures of streptococci isolated from gastric ulcers in man, dog, cattle or sheep, when injected intravenously into dogs and rabbits, produced typical gastric ulcers in a large percentage of the experiments. Whether the chronicity of this type of ulcer is due entirely to the streptococcus infection or the combined action of the streptococci and the corrosive action of the gastric juice has not been proven experimentally. Sippy (17) has shown in his treatment of human gastric ulcer, that the gastric acidity appears to play an important rôle in establishing a chronic ulcer. In neutralizing the gastric juice by constant administration of alkalies he has effected a cure for a large number of chronic gastric and duodenal ulcers.

Steinharter (18) claims to have produced gastric ulcers quite uniformly in rabbits, by intravenous injections of 24-hour old broth cultures of B. coli agglutinated with 0.3 per cent hydrocloric acid for 24 hours. One cubic centimeter of the 0.3 hydrochloric acid is added to 2.5 cc. of the 24-hour old broth culture. The agglutinated B. Coli are washed with sterile normal salt solution and injected into the ear vein of the rabbit.

METHODS

1. Thyroid feeding

The conflicting reports of Wolpe, Nielsen and others suggested the possibility of producing hyperacidity and hypersecretion of gastric juice in Pavlov-pouch dogs by feeding thyroid. Although Carlson (19) has shown conclusively that it is impossible to produce all the typical symptoms of exophthalmic goiter in dogs by feeding desiccated thyroids,

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the work was repeated with Pavlov-pouch dogs to determine the effects upon the gastric juice. Two dogs weighing 2.5 and 4 kilos, respectively, were fed 10 grams of Armour's desiccated thyroid daily. This thyroid feeding covered a period of two weeks. The collection of gastric juice was begun one hour before feeding and continued for one hour after feeding. The controls were made by feeding the same kind and quantity of meat in the absence of the thyroid. A series of controls were obtained before feeding the thyroid, another after the thyroid feeding was stopped.

2. Production of gastric and duodenal ulcers

a. Attempts with B. coli. In this series of experiments, the work of Steinharter was repeated on 18 rabbits and 3 dogs. The 24-hour old broth cultures of B. coli were agglutinated with 0.3 per cent HCl for 24 hours, using 1 cc. of the 0.3 per cent HCl to 2.5 cc. of the 24-hour old culture. The agglutinated bacteria were washed twice with sterile salt solution and injected intravenously into the animals. Five strains of B. coli were used.

Strain 1 was isolated from the normal faeces.

Strain 2 was isolated from a case of cholecystitis.

Strain 3 was grown on media containing normal salt.

Strain 4 was grown on media containing normal salt.

Strain 5 was a sub-culture of Strain 4.

Twelve rabbits were each given an injection of the growth from 5 cc. of a 24-hour old broth culture of B. coli agglutinated as above. Four of these rabbits were injected with Strain 4, four with Strain 3, and four with Strain 5.

Four rabbits were each given an injection of the growth from 10 cc. of a 24-hour old broth culture of B coli agglutinated as above. Two of these four rabbits were injected with Strain 1, and the other two with Strain 2.

Two rabbits were each given an injection of the growth from 10 cc. of a 24-hour old broth culture of B. coli which had not been agglutinated. One of these two rabbits was injected with Strain 1, and the other with Strain 2.

b. Ulcers produced by streptococcus. I followed Dr. Rosenow's technique in producing gastric and duodenal ulcers. My experiments, however, were chiefly on Pavlov-pouch dogs. In the first experiment the ulcer was produced by streptococci which Dr. Rosenow had isolated from a duodenal ulcer in a child. Later, at Dr. Rosenow's sug-

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gestion and under his supervision, the streptococci were isolated from the gastric ulcers in sheep and cows. The best results were usually obtained by isolating streptococci from the muscular coats of an ulcer which had undergone very little healing. By this method the possibilities for contamination were minimized. The dogs were injected intravenously with the growth from 40 to 60 cc. of the ascites dextrose broth, the dose depending upon the size of the dog.

In three dogs an attempt was made to produce an ulcer in the pouch by injecting the streptococcus directly into and beneath the mucosa. This resulted in the immediate production of a pocket in the submucosa containing streptococci. To make sure of the presence of an ulcer as the result of the injection the following examination was made:

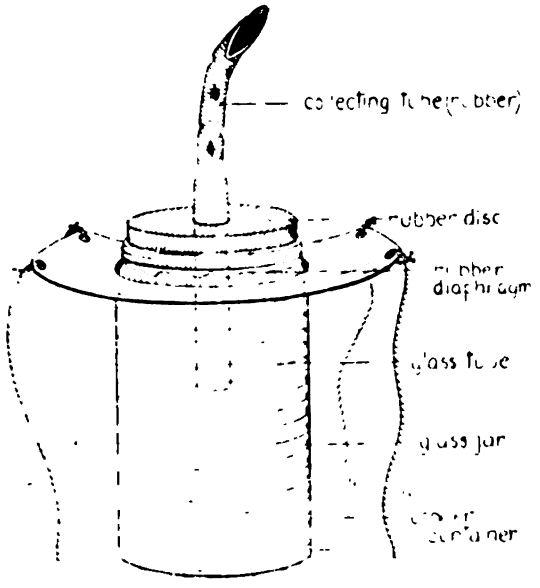


Fig. 1. Apparatus used in collecting gastric juice.

A small glass test tube with a movable mirror attachment at the bottom was introduced into the Pavlov-pouch of the dog. By throwing light into the tube sufficient illumination was obtained to enable one to examine the mucosa of the pouch. Three days after this injection there was no evidence of an ulcer by the examination described above.

c. Collection of gastric juice. The juice was collected in a small glass cup about 8 cm. high with a round base 5 cm. in diameter and a round top of 4 cm. in diameter. The cup was held in position by a slightly larger copper cup which fitted closely around the other cup. The copper cup had two small handles through which cords were passed and tied on the back of the dog. In this way the glass cup was held tightly in position against the abdomen of the dog. The glass cup had a tin screw top and lined with a thin rubber sheet which prevented any

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leakage. A hole was bored in the center of the cover just large enough for the glass tube from the pouch to pass through it. By making the hole in the rubber sheet smaller than that in the tin cover the glass tube which drained the juice from the pouch was made to fit practically water tight. By means of such a mechanism (see fig. 1) the dog could walk about or lie down in his cage without spilling any of the juice. This method could not be employed if a dog persisted in lying on its back for in that case the gastric juice would leak around the tube and be absorbed by the bandage.

3. Manner of collection

The collection of gastric juice was made in the morning, care being taken to remove water from the cage at least one hour before beginning an experiment. In every instance but one (in that case the collection was begun one hour before feeding and continued one hour after feeding without removing the cups) the gastric juice was collected two hours before feeding and the volume, total acidity and free acidity was determined. The sample of juice collected before feeding will be called the "continuous secretion." The dogs were then fed a standard meal of 250 to 350 grams of ground, boiled beef moistened with water. The juice was collected at one hour intervals for two hours after feeding. The acidity was determined by titration with $\frac{N}{10}$ NaOH and using dimethyl amino azobenzene and phenolphthalein as indicators for the free and the total acidity respectively. The experiments were made on the dogs four to six times a week for at least two weeks and in some cases as long as four to eight weeks. After a sufficient number of control experiments were obtained they were given an injection of streptococci into the saphenous vein. Whenever a sufficient amount of streptococci were available, normal control dogs were injected with the same amount of streptococci that were given to pouch dogs. The control dogs were usually posted from 24 to 48 hours after an injection. If no ulcers were present in the normal dogs, the Pavlov-pouch dogs were later given a second injection. In one dog three injections of streptococci were made. The gastric juice was studied for varying lengths of time after the injection, that is from 2 to 8 weeks, in the same manner as it was studied before. The dogs were killed and then autopsied.

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RESULTS

1. Thyroid feeding

The thyroid feeding experiments were discontinued at the end of the second week because there was no indication of either hyperacidity or hypersecretion. In both dogs on the contrary, there was a tendency toward depression of the acidity and the rate of secretion as is shown in Table 1. The dogs were in perfect health throughout the experiment, and from all appearances the thyroid had no toxic effects; they ate readily and so far as could be judged there were no gastro-intestinal disturbances. The acidity and volume of the gastric juice returned to normal a few days after the thyroid feeding was discontinued.

2. B. Coli

Of the eighteen rabbits injected with B. coli, negative results were obtained in all but four rabbits. In the rabbits where lesions were produced, all were injected with Strain 4. The results of the injection with Strain 4 are as follows:

Rabbit I died in less than four hours after the injection. Autopsy performed immediately after death. The stomach was perforated at the fundus. There were ulcers and hemorrhages near the pyloric end. The intestines were quite normal. The ulcers were not the typical round ulcers. The mucosa appeared to be sloughed off in places. The muscular coats were normal so that it was deemed useless to attempt to make a pure deep culture of the ulcerated area.

Rabbit II died in less than 24 hours after the injection. Autopsy: There was an ulcerated area near the fundus; intestines showed signs of hemorrhage.

Rabbits III and IV had hemorrhages in the stomach and slightly hemorrhagic areas in the duodenum. Both were killed 48 hours after the injection. No typical round ulcers were found.

In all three dogs the strain (1, 2, 3) of B. coli proved highly toxic; the dogs died in less than 24 hours and no specific lesions were found. In one of the dogs there was considerable hemorrhage in the duodenum and stomach.

3. Production of gastric ulcer by injection of streptococci

Ulcers were produced by injections of streptococci isolated from ulcers in man, sheep, cattle and dogs. The ulcers produced may be divided into the "acute" and "chronic" type of ulcer. The term

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TABLE I

The influence on the secretion of gastric juice, of excessive thyroid feeding experimental "Hyperthyroidism."

CONDITION OF DOG	NUMBER OF EXPERIMENT	VOLUME			TOTAL ACIDITY			FREE ACIDITY		
		Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.
Dog I.										
One hour before and one hour after feeding 250 grams meat	20	18	8	13.15	0.4922	0.3737	0.4183	0.4649	0.3281	0.3644
Feeding 250 grams meat and 10 grams of thyroid. One hour after and one hour before feeding	10	16	3.3	8.2	0.3463	0.1915	0.3068	0.3646	0.1459	0.2674
Stopped feeding thyroid, one hour before and one hour after feeding	12	14	8	10.8	0.4922	0.0820	.05481	0.4102	0.0450	0.3126
Dog II.										
One hour before and one hour after feeding 300 grams meat	10	13	7	9.09	0.4010	0.1820	0.3047	0.3646	0.1361	0.2532
Feeding 300 grams meat a and 10 grams of thyroid. One hour before and one hour after feeding	12	13	2	6.5	0.3646	0.0000	0.1139	0.3281	0.0000	0.0625
Thyroid feeding stopped. One hour before and one hour after feeding	8	16	5	10.6	0.4193	0.1704	0.3281	0.3593	0.0656	0.2372

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acute as applied here is an ulcer which is present in an active progressive state ten days to three weeks after an injection. The chronic ulcer is one which is active five to eight weeks after an injection. With one exception the ulcers resulting from the streptococcus injection lodged in the Pavlov-pouch. In Dog 7 a chronic ulcer was formed in the duodenum, about 8 cm. from the pylorus. Twelve normal Pavlov pouch dogs were examined for gastric ulcers but no ulcers were present. Spontaneous gastric ulcers were found in two Pavlov-pouch dogs not injected with bacteria, and in one Pavlov-pouch dog with complete pancreatectomy. At atopsy cultures were made from most of the ulcers and pure cultures of streptococci were isolated. The virulence of the isolated streptococci was demonstrated in two cases by injections into two dogs. Gastric ulcers with hemorrhages of the stomach followed the injections, and both dogs died in less than 24 hours. Gross and microscopic examinations were made in every case and active progressive ulcers were demonstrated.

Ulcers were produced in eight cases out of the six normal dogs and eight Pavlov-pouch dogs. Positive results were obtained therefore in 57 per cent of the experiments, seven of those successful attempts were in Pavlov-pouches.

DISCUSSION

The thyroid feeding experiments are not sufficient in number to warrant a conclusion as to the cause of the depression in volume and acidity of gastric juice during the first hour after eating. Further work should be done to determine the cause of the depression and whether or not the depression involves only the secretion of the appetite gastric juice or the entire secretion curve. To determine this it would be necessary to follow out the secretion during the entire course of digestion. The depression is not permanent as is shown by the rapid return to normal acidity and secretion rate when the thyroid feeding was stopped.

The experiments with B. coli do not confirm the work of Steinharter. In only one series of experiments was there any indication that intravenous injections of B. coli in rabbits (by Steinharter's method) would produce gastric ulcer. Five different strains of B. coli were used, but only one strain appeared to be toxic to the rabbits' stomach and gastric *lesions* were produced in every one of the four rabbits injected with that strain (Strain 4). There is in all probability a difference in the virulence and specificity of the toxins produced by B. coli. But the

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gastric lesions resulting from intravenous injections of B. coli are not the typical ulcers which can be produced by streptococci.

The productions of gastric ulcers in dogs by intravenous injections of streptococci which were isolated from gastric ulcers in man, dogs, cattle and sheep, is a confirmation of Rosenow's work. The fact that the ulcers lodged (in the majority of cases) in the Pavlov-pouch of the dogs, demonstrate many points of interest which undoubtedly are of some clinical significance. The intensity of the movements and contractions of the Pavlov-pouch is about as vigorous as in the fundus of the main stomach. Since there was no obstruction in the pouch to alter the motor mechanism, the chronicity of the ulcers must be explained on some other basis. The acidity of the gastric juice was not materially increased in any of the dogs and only two of the dogs with ulcers showed a hypersecretion.

One is inclined to lay less stress upon the factors of acidity, secretion and mechanical obstruction when we see active chronic ulcers produced in the Pavlov-pouch. In the pouch the motility is less than in the pylorus, there is practically no mechanical irritation, and the gastric juice is constantly being drained from the pouch. Since the mechanical factors and the corrosive action of the gastric juice is practically eliminated, the chronicity of the ulcer must depend primarily upon the virulence of the organism producing the ulcer. This is further demonstrated in the Pavlov-pouch operation. There is considerable destruction of the mucosa in a Pavlov operation; but since the necrosed mucosa is not infected, healing takes place in a few days in spite of the "corrosive action" of the gastric juice. The production of the gastric ulcers and the chronicity of the ulcer is dependent primarily upon the virulence of the streptococci infection. The haematogoneous origin of the infection is further demonstrated in the unsuccessful attempt to produce chronic ulcers by local injections of streptococci into the submucosa of the Pavlov-pouch.

The striking variations in the secretion rate and acidity which each dog showed after a test meal will probably explain some of the conflicting clinical data. The results were obtained from seven dogs with gastric and one with a duodenal ulcer (figs. 2 and 3). There was no change in the concentration of the acidity which indicated the presence of an ulcer in any one of the dogs. The acidity ranged from 0.0000 per cent to 0.55 per cent both before and after the production of the ulcer. There was a depression in the acidity of the gastric juice in Dog III. In two of the dogs (Dogs IV and II, Table II) there was a tem-

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porary continuous hypersecretion and a hypersecretion after eating but the acidity was about normal.

In Dog II there was a hypersecretion in spite of the fact that she had developed distemper a few days after the injection. Distemper in a majority of cases depresses the acidity and secretion. Why only two of the eight dogs should develop a hypersecretion we are not at present able to satisfactorily explain.

This hypersecretion may have been the result of an increased sensibility of the gastric mucosa either produced locally or as a result of the absorption of toxins from the ulcer and a stimulation of the vagus fibers. In gastric ulcers involving extensive areas of mucosa it is quite conceivable that a hypersecretion might result from the formation and absorption of gastrin. To determine whether the hypersecretion is due to a local stimulation, or reflex, it would be necessary to perform a series of similar experiments on dogs with Heidenham pouches in which the vagus fibers going to the pouch have been severed.

CONCLUSIONS

1. Feeding excessive amounts of desiccated thyroid depresses the rate of secretion and concentration of acidity in the gastric juice during the first hour after feeding.

2. The results of Steinharter ("The production of acute ulcers in rabbits by intravenous injections of B. coli") have not been confirmed.

3. Gastric and duodenal ulcers can be produced in dogs by intravenous injections of streptococci isolated from gastric ulcers in man, dog, sheep and cattle. This is a confirmation of the work of Rosenow.

4. There is no "hyperacidity" in the gastric juice following the experimental production of gastric ulcers.

5. Gastric and duodenal ulcers may or may not result in a continuous hypersecretion together with a hypersecretion after eating.

I wish to thank Dr. Rosenow under whose supervision the bacteriological work was carried out; and Dr. Carlson for his suggestions.

PROTOCOLS

Dog I.

Experiments were begun June 8 and continued until August 19, 1915.

Two injections of streptococci, which I isolated from ulcers in sheep were made directly into the mucosa and submucosa of the Pavlov pouch. No ulcers resulted from the injection.

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July 19. Injected the growth from 40 cc. of ascites-dextrose broth of streptococci isolated from an ulcer of a cow.

Injected a similar dose into a normal dog of about the same weight. Following the injection of streptococci, both in the normal and in the Pavlov dog; the dogs vomited a bile colored fluid. Depression followed the vomiting and lasted for 5 to 6 hours. The day after the injection the dogs were quite active.

July 21. Posted the normal control dog and found an ulcer in the pyloric end of the stomach. The ulcer was about 5 mm. in diameter.

The Pavlov dog was in perfect condition during the entire experiment.

August 20. She was chloroformed; autopsy showed her to be quite normal except for a single elongated ulcer in the small pouch. The ulcer was 2 cm. long and 4 mm. in the widest portion. The edges of the ulcer were rounded and slightly undermined. The base was smooth and fibrous. The extremities of the ulcer were actively progressing. The edges of the extremities were elevated and hyperaemic.

Dog II.

Experiments covered a period of 44 days, beginning April 14 and ending May 27. Two weeks were allowed for the dog to recover from the effects of the Pavlov operation before beginning the experiments.

May 7. Injected streptococci, isolated by Dr. Rosenow from a gastric ulcer in a hog, directly into the submucosa of the small pouch.

May 9. No ulcer was found at the site of injection. Examination was made by introducing a small test tube with a mirror inside, into the pouch. By this method one could carefully study the mucosa of the pouch.

May 18. I injected intravenously with the growth of 40 cc. of ascites-dextrose broth of a strain of streptococci which I isolated from a gastric ulcer in a sheep.

May 20. She ate very heartily but secreted slightly more juice than her maximum secretion up to this time. The juice was blood tinged.

May 21. The dog does not eat very much and secreted a few cubic centimeters of blood tinged juice.

May 24. Dog shows beginning distemper. She refused food and water. In the absence of food or water there was a continuous hypersecretion with an acidity of approximately 0.3 per cent. The stomach was aspirated but no food was presented.

May 25. Condition about the same as May 24.

May 26. Same as May 25 but more mucous was secreted.

May 27. Only mucous was secreted.

Killed her. Autopsy: Slight lesion in the heart, all the other organs are normal. A large irregular ulcer, 1.5 by 2 cm. was found a little to one side of the suture line in the small pouch. The mucosa was completely necrosed but had not sloughed off as yet.

Culture showed mainly streptococci with a few B. coli. Microscopic examination showed active progressive ulcer which had extended into the muscular coats. There was a slight scar tissue formation. Leucocytic infiltration had extended into the muscular coats.

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Dog III

The experiments on this dog covered the period from April 9 to August 26.

May 6. Injected streptococci, isloated from gastric ulcer in a sheep, directly into the submucosa. No ulcer was produced.

May 19. Injected the growth from 10 cc. of ascites-dextrose broth of streptococci, isolated from a gastric ulcer in a sheep, directly into the submucosa. No ulcer was produced.

May 23. Injected the growth from 60 cc. of ascites-dextrose broth of a 24-hour culture of streptococci isolated from an ulcer in a sheep. The same dose was injected into another Pavlov pouch dog and killed a week later. No ulcer was found.

One normal dog and a normal rabbit were injected with the same dose and 58 hours later were killed. The dog had a single small ulcer in the pyloric end of the stomach. The rabbit had a small ulcer near the fundus of the stomach.

Dog VII, immediately after the injection, had violent vomiting movements which were followed by marked weakness. The dog recovered completely by the following day.

The dog appeared in perfect health with the test meals and laboratory treatment up to the time she was killed (August 27).

Autopsy: All the organs were normal; the stomach, including the Pavlov pouch, was quite normal.

There was a single round ulcer about 1.5 cm. in diameter located 6 cm. from the pylorus in the duodenum. The margins of the ulcer were elevated and undermined. The base of the ulcer was hard and smooth.

Dog IV.

Operated for Pavlov pouch about six months previous to injection. She was injected February 16, with the growth of from 25 cc. of ascites-dextrose broth of a strain of streptococcus isolated by Dr. Rosenow from a duodenal ulcer of a 12 year old child. There was vomiting and great weakness following the injection. This condition lasted for about 2 hours.

February 17. The dog seems fairly well, eats heartily but secretes practically no juice during the first 2 hours after feeding. This condition continued for one week after the injection. During that time the juice (which was collected for one hour before and two after feeding) consisted of a thick bloody mucous practically free from acid.

February 25. A clear juice was secreted; both before and after feeding. Following the sudden change from the bloody mucous secretion to the clear juice that resulted in a slight increase in the average volume of gastric juice amounting to 3.1 cc.

There was a continuous hypersecretion which lasted for six days and then returned to normal. The dog continued in an apparently normal condition until March 1, when she began to show muscular tremors, slight stiffness of the joints and great weakness.

March 1. The dog died and was autopsied immediately.

Autopsy: There was hemorrhage in the small intestine, and a coffee ground colored fluid in the stomach. The mucosa of the stomach was normal except

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for a round pouched out ulcer in the pouch, 2 cm. from the suture line; the ulcer was 12mm. in diameter. The margins of the ulcer was thickened and hyperemic. The center of the base of the ulcer showed scar tissue. Microscopic examination —There was marked leucocytic infiltration which extended into the submucosa and muscular coats. Culture made by Dr. Rosenow showed a great many streptococci and a few B. coli.

The streptococci was injected into two other dogs and produced ulcers in both dogs. The dogs died in less than 24 hours of hemorrhage of the intestine and stomach.

Dog V

Pavlov pouch operation was performed 2 weeks before beginning the experiments. The experiments covered a period of three months, beginning November 24 and ending February 24.

January 6. The dog received an injection of streptococci, isolated from an ulcer by Dr. Rosenow. Control experiments showed the strain to be of a low grade of virulence.

January 28. Streptococci, which were isolated from a gastric ulcer, were injected intravenously. Following the injection the dog vomited and appeared very weak for several hours.

January 30. The dog is lame—the hind legs are somewhat stiffened.

February 22. Noted that food passed through the main stomach into the pouch.

Febuary 24. The dog was killed and an ulcer was found at cap of the small pouch. The ulcer had perforated so that there was a direct communication between the large and small pouch.

Cultures showed many streptococci, a few B. coli. Microscopic and gross examination showed an active progressive ulcer. The ulcer was about 6 mm. in diameter. The margins were elevated and slightly undermined.

Dog VI

Experiments covered a period of 2 weeks, beginning March 31 and ending April 15. The data is given in the table. No injection of streptococci was made in the dog.

April 10. The dog began to show signs of distemper.

The main stomach was markedly hemorrhagic.

The pouch appeared quite normal except for a small round, deep ulcer situated in the middle of the pouch and some distance from the suture line. The base of the ulcer showed some fibrous tissue. The edge was slightly undermined showing the ulcer to be quite active and progressing. This was a spontaneous ulcer evidently of streptococci origin as culture showed practically all streptococci.

In the dog the ulcer was evidently present from the time the experiments were begun. There was no evidence of a continuous hypersecretion or hyperacidity. The case is similar to that of Dog III. Both dogs developed sniffles and both dogs had ulcer in the pouch—in the one case there was no evidence of continued hypersecretion (Dog IV) while in the other there was a temporary continuous hypersecretion (Dog III).

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Dog VII

Operated July 9, 1915; completely healed by July 13. Experiments were begun on her July 13 and continued until August 9, when she was killed. She appeared perfectly normal and ate readily until July 23. She refused food for 2 days but by July 29 she had recovered and was again quite normal.

August 4. She began to show symptoms of distemper.

August 5–8. The symptoms of distemper are more prominent. Tried to "force feed" her but she could retain nothing on her stomach.

August 9. Killed. Autopsy: Heart, lungs, kidney and spleen normal. Found partly healed round ulcer (about 6 mm. in diameter) in the small pouch. The main stomach had one small ulcer which was almost healed. There was an acute gastritis of the main pouch; the small pouch was quite normal except for the ulcer. The ulcer in the small pouch was active and progressive. The margins were more or less slightly undermined and infiltrated.

RESULTS

The acidity and rate of secretion of juice with gastric ulcer

In Table II is given the data on the dogs in which gastric ulcers were produced, either experimentally or spontaneously, together with the data which is typical of a number of the dogs in which no ulcers were produced and which are designated normal dogs. One can readily see from an examination of the data for "normal dog" that there is considerable variation in the acidity and rate of secretion of individual dogs and in the same dog even when a standard meal is given. In the table including the work done on the dogs with ulcers, the same wide variations can be seen both before and after the production of the ulcers. There were only two dogs in the series in which there was any indication of a change in the nature of the juice. Dogs IV and II both showed a tendency toward continued hypersecretion which lasted for a few days and then returned to normal. In *no* case was there any change in the acidity of the gastric juice which could be considered a "hyperacidity." In Dog III there was a slight depression in the acid concentration. There were variations and fluctuations in the rate and acidity of the gastric juice during the course of the experiments which one is bound to consider as normal variations.

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TABLE II

The gastric juice in experimentally produced gastric and duodenal ulcers

CONDITION OF DOGS	NUMBER OF EXPERIMENTS	VOLUME			TOTAL ACIDITY			FREE ACIDITY		
		Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.
Dog. I, Normal										
Two hours continuous secretion	15	4.5	1 drop	2.5	0.0450	0.0000	0.00478	0.0364	0.0000	0.0024
First hour after feeding	15	5	0.5	2.84	0.2462	0.0458	0.1415	0.2280	0.0000	0.1080
Second hour after feeding	10	7	1.5	4.5	0.4375	0.1368	0.2907	0.4010	0.0547	0.2409
Dog I, Gastric Ulcer										
Two hours continuous secretion	15	5	0	2	0.0912	0.0000	0.0072	0.0364	0.0000	0.0024
First hour after feeding	14	3.5	1	2	0.2188	0.0000	0.1324	0.1915	0.0000	0.0863
Second hour after feeding	14	6.5	1	3.2	0.4375	0.820	0.3034	0.4010	0.0364	0.2679
Dog II, Normal										
Two hours continuous secretion	16	4	1	2.28	0.2462	0.0000	0.0176	0.2006	0.0000	0.0143
First hour after feeding	15	7	2	3.45	0.3737	0.0000	0.1778	0.3372	0.0000	0.1432
Second hour after feeding	11	7	1	4	0.4010	0.1641	0.3320	0.3554	0.1276	0.2942
Dog II, Gastric Ulcers										
Two hours continuous secretion	8	10	2.5	6	0.3646	0.0000	0.2301	0.3190	0.0000	0.1892
First hour after feeding	5	9	2	4	0.3281	0.2097	0.2712	0.2736	0.1550	0.2077
Second hour after feeding	4	13.2	2	7	0.4284	0.2097	0.3524	0.4010	0.1550	0.3129
Dog III, Normal										
Two hours continuous secretion	25	11	0.5	2.03	0.4193	0.0000	0.0550	0.3919	0.0000	0.0460
First hour after feeding	14	10	2.25	5.7	0.4375	0.2462	0.3365	0.4102	0.2006	0.3043
Second hour after feeding	10	8	1.5	5.1	0.5287	0.3919	0.4474	0.5014	0.3646	0.4206
Dog III, Duodenal Ulcer										
Two hours continuous section	26	4.5	0.5	2.4	0.2644	0.0000	0.0155	0.2371	0.0000	0.0102
First hour after feeding	35	6.5	1.5	3.2	0.3463	0.0364	0.1704	0.3180	0.0000	0.1375
Second hour after feeding	24	7.75	1	3.8	0.4193	0.1459	0.3176	0.3828	0.0547	0.2742

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TABLE II—Continued

CONDITION OF DOGS	NUMBER OF EXPERIMENTS	VOLUME			TOTAL ACIDITY			FREE ACIDITY		
		Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver
Dog IV, Normal										
Two hours continuous secretion.....	14	11	2	3	0.4193	0.0000	0.0656	0.3737	0.0000	0.011
One hour continuous secretion combined with one hour after feeding.....	20	13	7	9.09	0.4010	0.1820	0.3047	0.3646	0.1361	0.253
Two hours continuous secretion combined with two hours after feeding.	15	25	5	16.7	0.4570	0.2372	0.3593	0.4272	0.1640	0.31
Dog IV, Gastric Ulcer										
First Week										
Two hours before and two hours after feeding.....	7	6	1	3.2	0.0000	0.0000	0.0000	0.0000	0.0000	0.00
Second Week										
Two hours before and two hours after feeding.....	6	32	11.5	19.6	0.3737	0.2553	0.2904	0.3463	0.1368	0.24
Two hours continuous secretion.....	6	11.5	4.5	8.2	0.3919	0.1915	0.2862	0.3463	0.1368	0.25
Dog V										
One hour before and one hour after feeding.....	20	18	8	13.15	0.4922	0.3737	0.4183	0.4649	0.3281	0.36
One hour before and one hour after feeding.....	12	14	8	10.8	0.4922	0.0820	0.3481	0.4102	0.0450	0.28
Dog VI, Spontaneous Ulcer										
Two hours continuous secretion.....	8	4.5	0.5	2.1	0.1294	0.0000	0.0258	0.0642	0.0000	0.012
One hour after feeding.....	8	7	2.5	4.1	0.3007	0.1550	0.2081	0.2644	0.1276	0.177
Second hour after feeding.....	6	7	0.5	3.4	0.4193	0.1641	0.2780	0.4041	0.1185	0.243
Dog VII, Spontaneous Ulcer										
Two hours continuous secretion.....	10	6	0	2.1	0.1820	0.0273	0.1194	0.1276	0.0000	0 29
First hour after feeding.....	10	10.5	1	4.9	0.4102	0.2553	0.3208	0.3463	0.1820	0.266
Second hour after feeding.....	10	12.5	1.5	6.2	0.4740	0.3281	0.4128	0.4465	0.2462	0.376

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TABLE II—Continued

CONDITION OF DOGS	NUMBER OF EXPERIMENTS	VOLUME			TOTAL ACIDITY			FREE ACIDITY		
		Max.	Min.	Aver.	Max.	Min.	Aver.	Max.	Min.	Aver.
Normal Dog I										
Two hours continuous secretion	14	6	1	2.3	0.2006	0.0000	0.0196	0.1276	0.0000	0.0175
One hour after feeding	14	9	2	5.3	0.4010	0.0734	0.2636	0.3646	0.0547	0.2195
Two hours after feeding	14	12	2.5	6.1	0.4508	0.3281	0.4001	0.4284	0.3007	0.3685
Normal Dog II										
Two hours continuous secretion	5	6	4	4.04	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
One hour after feeding	5	7	4	5.4	0.2736	0.1276	0.1987	0.2553	0.0912	0.1641
Second hour after feeding	5	5	3.5	4	0.3007	0.2097	0.2507	0.2736	0.1820	0.2256
Normal Dog II										
One hour before and one hour after feeding	10	14	4	7.6	0.3737	0.1276	0.2673	0.3281	0.0364	0.2066

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BILE PIGMENT METABOLISM

I. Bile Pigment Output and Diet Studies

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In the following communications we propose to show that the bile pigment secretion can be influenced at will by modification in the diet. This means probably that the liver has a *constructive* function in forming bile pigments as well as the accepted eliminative function which depends on the destruction of red cells containing hemoglobin. The statement that bile pigment elimination may be influenced by dietary conditions is proved conclusively for dogs, and there is every reason to suppose that it is true for other animals.

We realize that the above statements are contrary to the accepted views of physiologists, and will require very convincing proof which we submit in detail below. The generally accepted theory covering the life history of the bile pigments may be sketched somewhat as follows. Degeneration of red cells frees hemoglobin which is brought to the liver and there changed to bile pigments which are excreted as waste products into the intestine. Here the bile pigments are reduced to urobilin or stercobilin some of which may be absorbed and returned to the liver and again thrown out in the bile or destroyed. Some of this urobilin may escape the liver and appear in the urine, especially when the liver is not functioning normally.

We hope to add several factors to this relatively simple equation which may throw more light on the functional capacity of the liver as well as the cycle of pigment metabolism in the body. We have established (12) the fact that hemoglobin can be rapidly changed to bile pigment in the body circulation outside of the liver. McNee (5) has recently confirmed this observation. We (4) have also shown that the pleural and peritoneal cavities can rapidly transform hemoglobin into bile pigment. We believe that this extrahepatic transformation of hemoglobin into bile pigment may be more important than is generally

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supposed, particularly in diseased conditions associated with icterus or hemoglobinemia.

We (11) have recorded the observation that an Eck fistula dog with obstructed common bile duct will develop icterus to a much less degree than a normal dog with obstructed duct. It has been proved that an Eck fistula liver is smaller than a normal liver and has less functional capacity (13 and 14). The suggestion is obvious that the production of bile pigments is in part due to the functional activity of the liver and not solely to the hemoglobin destruction. In this manner were we lead into a study of bile secretion and its many difficult problems.

An immense amount of work has been done upon the secretion of bile, and it is fair to say that most of it was not done in a critical spirit but apparently with the idea of proving that some one or more substances were cholagogues. Stadelmann (7 and 8) and his co-workers are among the few who emphasize the normal variations in bile flow in dogs and the extreme care necessary in drawing any conclusions from small fluctuations in bile excretion. Their experimental observations are carefully made, suitable controls are furnished, and great care is used in the analysis of figures. Their work is to be recommended as an example for all workers in this difficult field.

"Bile circulation" has been definitely established—Stadelmann (9). This means that the *bile salts* are poured into the intestine, partially absorbed from it and again excreted in the bile. This fact comes in with the observation that bile or bile salts are active cholagogues, in fact the only substances upon which there is agreement among experimental workers. Among the dozens of other drugs used as cholagogues one can pick out any single drug and find in the literature several workers who claim to show that it is a cholagogue and again the same number who claim as proven from their work that it has no cholagogue action. Almost all of the conservative workers agree that whole bile or bile salts alone give definite acceleration to the flow of bile.

"Bile circulation" for the *bile pigments* has been claimed but never demonstrated. Stadelmann (9) says that a very small amount of bile pigment *may* be absorbed from the intestine but there is no definite proof for this, and he leaves the question open. There is no question that the liver can pick bile out of the circulating blood and rapidly excrete it through the gall ducts. This applies to foreign bile as Wertheimer (10) showed by means of sheep's bile injected into dogs. This, however, does not prove that bile pigments can be absorbed from the *intestine* and excreted again in the bile as is assumed by some writers.

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Hemoglobin injected into the blood stream, peritoneum or subcutaneous tissues will cause a rise in bile pigment output from the liver. Stadelmann and his pupils submit the best experiments on this point, but they do not claim that it is quantitatively eliminated as do Brugsch and Yoshimoto. The published data of Brugsch and Yoshimoto (2), Brugsch and Kawashima (3) do not establish their claims that hematin is quantitatively eliminated as bile pigment. But grant for the sake of argument that hematin *is* quantitatively eliminated as bile pigment, one is surprised at their argument therefrom that hemoglobin is the source of the bile pigment, and from the bile pigment one can compute the life cycle of the red cells! There may be a dozen substances which may be quantitatively or partially changed into bile pigments or the liver cells may be capable of building up bile pigments from various "building stones."

Some work has been done upon the bile pigment excretion as influenced by the injection of hemoglobin, bile and bile pigments, also various poisons known to injure the liver or destroy red blood cells (Stadelmann, Brugsch, Wertheimer, etc.). So far as we know no worker has followed the curve of bile pigment excretion with suitable control of general condition and weight of dog, hemoglobin estimation and red cell counts, the presence or absence of pigments in the urine and above all, the diet. For the present we shall confine our attention to the bile pigment output with occasional notes concerning the volume of bile flow.

At the beginning of this work about three years ago it seemed quite necessary that the bile fistula dogs should be maintained as near to a normal healthy condition as possible. Considerable time was spent upon this point, and every sort of ration was tried out, mixtures given with fresh bile and dried bile, raw liver and cooked liver, raw and cooked meat, milk, raw eggs, butter, fats, etc. We do not wish to dwell upon negative results, but will give only a review of our positive results.

We are convinced from our work that bile is a necessary life factor for a dog fed upon any common mixed diet. There are statements in the literature Albu (1), Ransom (6) that bile secretion is not essential to health in man, but we are sceptical of such reports as careful autopsy records are not submitted. We are convinced from our series of over twenty animals that it must be very unusual for a dog to be able to survive on any ordinary diet if the bile is *completely* excluded from the intestine. In our experiments, the bile which the animal may lick from its fistula during the night is not able to maintain normal equilibrium or anything approximating it.

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We wish to point out again that the common bile duct can reestablish its lumen after double ligation with silk and resection of about 1 cm. of the duct. We have reported such cases in another paper (11), and note also that this can happen in a dog with a bile fistula. A tiny fistulous tract may establish itself between the cut ends of the common duct and allow the escape under pressure of a small amount of bile into the duodenum. One such dog is included in our series of simple bile fistulas. This point must be kept in mind and the stools watched very carefully for stercobilin and at autopsy a very careful search made at the site of the section of the common bile duct. When a dog with a permanent bile fistula on an ordinary diet holds his normal weight and condition, we believe that this possibility should be considered and excluded or not by examination of the feces. Only a very small amount of bile seeping into the duodenum is required to completely change the clinical picture of wasting and general malaise to one of health and activity. Study of these repaired common ducts at autopsy shows how small an amount of bile introduced at this point is necessary for health. Introduction of bile by stomach tube, however, has no such favorable effect.

Fresh bile (pig) was tried in two bile fistula dogs with poor success. It was given once or twice daily by stomach tube. This did not prevent the usual loss of weight on a mixed diet. Recently we have had better results using fresh dog's bile mixed with the food, but some animals will not eat this mixture, and it does not have permanent effects when given once or twice daily in 25 to 50 cc. amounts by stomach tube.

Dried ox bile (two grams per day) was given in capsules to many of the dogs in our earlier experiments. This dried bile surely helps maintain a normal condition, but as a rule is not sufficient with a simple mixed diet, and most of these bile fistula dogs died after several weeks with the familiar picture of emaciation, intestinal disturbances, including much loss of blood, and stupor. Dried ox bile cannot be counted upon to replace in any satisfactory way the normal flow of bile, but it does some good in some instances.

Fresh pig's liver was tried as a diet following quite a series of unsuccessful bile fistulas which were fed bile in various ways. Marked improvement was noted with fresh liver forming a part of the diet, but the dogs soon refused to eat the raw liver. Cooked liver was then tried with the same success, and we feel very certain that bile fistula dogs can be kept in practically normal condition and weight equilibrium

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on a diet of cooked liver (pig or sheep). This statement must be slightly qualified as some dogs do not react as well as the majority: again the dogs' condition may remain perfect for months with a terminal loss of ground and intoxication. The diet used in the majority of experiments was the usual mixed diet of cooked meat, bones, and bread plus 100 to 200 grams of cooked liver as indicated in the charts.

That liver feeding in dogs with complete bile fistulae is of peculiar benefit, and may maintain them in a normal condition for weeks is pretty definitely established. This liver feeding is more efficacious in most cases than feeding fresh or dried bile, and is to be considered in the treatment of certain clinical cases. What particular chemical substance in the liver is responsible for this influence on the abnormal metabolism of bile fistula dogs? We hope to give an answer to this question in the near future.

METHODS

1. Operation and post-operative care

The operative procedure and care of the animals are very important factors in these experiments, and have not been sufficiently emphasized by many workers. The object should be to maintain the dog in as near to perfect condition as possible, and this is not easy.

All operations are done under ether-morphia anaesthesia, and strong, active, short-haired dogs of about thirty pounds weight are most suitable. An incision is made in the mid line, and the gall bladder dissected free from the liver. The common duct is freed, doubly ligated, and about 1 cm. between the ligatures is resected. The gall bladder is then pulled through a small stab wound in the right rectus close to the costal morgin, and fixed by silk sutures to the sheath of the rectus. The stab wound should be rather small, as a small fistula is desired. The gall bladder is then opened, and a small piece of rubber tubing about 1 cm. in diameter is pushed down into its lumen, and fixed here by two stay sutures. The median incision is closed as usual. No dressings are applied, as they only serve to irritate the skin, and the wounds heal very promptly. The tube in the gall bladder should be removed on the sixth or eighth day, and care should be taken that the tube drains freely, else a distinct icterus may result, and prolong convalescence or actually render the animal useless for further work. If there are no complications, the dog should be in good condition by the third week, and ready for bile collections by the method described below.

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A regular routine is very important in the experiments, and is a part of the care so necessary to keep the dogs in good condition. The dogs are allowed to exercise in a yard for about one-half hour in the morning. They are brought in for collection of bile about 10 a.m., and put up in the harness for a period of six or eight hours, during which time specimens are removed every two hours. The dogs are fed two hours after the start of the experiment each day. At the end of the observation they are turned into the yard to exercise about one hour, and then given a heavy feed and locked in their cages. Dogs and cages are kept very clean, and dogs are washed once or twice a week. Collections are made *every* week day, and this is important, because otherwise the fistula will narrow and obstruct the outflow, jaundice will supervene, and the general condition of the dog will suffer. Great variations in the bile pigment output will then occur. It is best to dilate the bile fistula occasionally but very gently. On Sundays the fistulas are drained by a catheter, but no collections are made. Under this régime, the bile collected will be perfectly clear, except for an occasional small shread of mucus, and at autopsy the larger bile passages will be smooth, pale and normal throughout except for slight dilatation.

2. Collection of bile

A small flexible rubber tube about 7 cm. long is passed into the bile fistula, and should fit accurately. This tube passes through the short stem of a glass funnel, and is fixed firmly in it. This serves two purposes —first, the funnel catches a little mucus which oozes from the edge of the fistula, and prevents its mixture with the bile secretion; second, it will show any escape of bile about the tube which never occurs in suitable fistulae, and serves to hold the tube firmly in place in the fistula. The glass funnel is held tight against the abdomen and fistula by a binder reinforced by metal about the funnel. The binder is held accurately in position by adhesive plaster over the dog's back, and a small rubber bag is fixed to the end of the glass funnel stem to catch all the bile. Wide cloth and webbing strips are passed under the thorax and forelegs to prevent the dog from lying down on the rubber bag and spoiling the collection. The dogs stand or sit on their haunches, and doze quietly a good part of the period of collection.

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3. Bile pigment estimation

One cubic centimeter of the bile to be analyzed is added to 49 cc. of the following solution (ethyl alcohol 95 per cent, 100 cc., nitric acid concentrated, 0.4 cc., and hydrochloric acid concentrated, 2 cc.) and mixed in a volumetric flask. The flask is shaken thoroughly, corked, and allowed to stand at room temperature about eighteen hours, when the readings are made. This solution turns bluish green, and reaches its maximum color in twelve to eighteen hours, and holds its intensity for twenty-four to forty-eight hours or longer. The solution is filtered through paper, and read in a colorimeter (Autenrieth-Königsberger as modified by Rowntree and Geraghty). The method is very simple and accurate to 0.01 mgm. of bilirubin.

The bile pigments in the serum or urine are estimated as follows. The fluid is made alkaline with a saturated solution of sodium carbonate and mixed with a 10 per cent solution of calcium chloride giving a voluminous precipitate containing the bile pigments. The precipitate is thrown down and washed repeatedly with distilled water by use of the centrifuge. The precipitate is finally dissolved in a measured amount of the nitro-hydrochloric acid alcohol solution, and allowed to stand at room temperature over night. The pigments are then estimated by the colorimeter.

For this work it is very desirable to have a permanent standard wedge for the colorimeter, and after many trials the best result was obtained as follows. A normal solution of very pure copper sulfate is treated with a few drops of a dilute watery solution of India ink. This suspension is not permanent unless fixed in some way. This is accomplished by a solution of agar-agar and gelatin which must be cleared with great care. Equal parts of the gelatin-agar solution and normal copper sulfate solution are combined while still warm, and poured into the standard wedge. The mixture of copper sulfate, ink, and gelatin-agar when it cools is permanent, and the wedge may be sealed with vaseline. Our standard wedge had been standardized against (a) pure bilirubin obtained from human gall stones, (b) bilirubin C. P., Kahlbaum, source of bile unknown, and (c) pure crystalline dog bilirubin. A table has been constructed so that knowing the colorimeter reading and the amount of bile in cubic centimeters, the bile pigment can be read off directly.

Table I (Dog 16–6) shows the output of bile and bile pigments by a dog in good condition on mixed diet plus cooked liver. The bile

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secretion varies in six hours from a minimum of 42 cc. to a maximum of 69 cc, and the bile pigments from 29 mgm. to 42 mgm. The two hour periods are seen to vary greatly, but there is no relation to food which is always given at the end of the first two hours. Bile is excluded from the intestine and stercobilin is absent. There are only traces of bile pigment in the urine. This bull dog was very fat when operated upon, and the initial loss of weight during the first month is in part due to this fact. It is noted that bile fistula dogs in the best possible

TABLE I

Normal dog—mixed diet and liver

DOG 16-6*	BILE								URINE BILE PIGMENTS TOTAL, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
										pounds	
September 16.....	17	16	19	52	7.4	9.8	16.6	33.8	trace	36.5	R.B.C. 5,632,000: Hb. 87 per cent: W.B.C. 7,600.
September 17.....	24	20	16	60	11.7	10.5	6.7	28.9	0	36.3	Stools clay colored; no stercobilin.
September 18.....				62				32.1	0	36.0	
September 20.....	16	18	21	55	11.2	15.8	9.7	36.7	0	35.5	
September 21.....	24	24	18	66	15.4	14.0	13.0	42.4	trace	36.0	
September 22.....	23	21	18	62	14.1	13.1	13.4	40.6	0	35.5	
September 23.....	20	22	27	69	12.5	11.6	12.2	36.3	+	35.8	Stools clay colored; no stercobilin.
September 24.....	13	25	19	57	6.4	12.7	11.4	30.5	+	35.5	
September 25.....				42				33.0	trace	34.5	
September 27.....	23	18	14	55	13.5	8.3	8.1	29.9	+	34.0	September 30. Hb. 98 per cent.
Average.....				58				34.4			

* Bile fistula operation September 2, 1915. Usual mixed diet plus 150 grams boiled sheep liver with morning meal.

condition will carry but very little subcutaneous fat. If the dog is lean and muscular at time of operation, it will lose only a little weight, but if very fat, the dog will lose several pounds after operation in spite of any care and apparent good health and appetite.

Table II (Dog 16–6) shows the same dog as Table I two months later when in apparently excellent condition. The dog was fed a mixed diet plus cooked liver and fresh dog bile. It is to be noted that the bile volume is much greater than in Table I (average per six hours—58 cc.) as compared with the average of 96 cc. We believe this chola-

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gogue action is wholly due to the fresh bile feeding. On the other hand the bile pigments are *lower* (average 28.6 mgm. per six hours) as compared with Table I (average 34.4 mgm. per six hours). This drop in bile pigment output we do not believe is due to loss in weight, as the dog seemed quite normal and had a good appetite. We will discuss this point more in detail later.

The animal seemed normal in all respects until December 5, when she vomited some food. The next day she developed muscular tremors and convulsions shortly followed by death. The intoxications which

TABLE II

Normal dog—mixed diet, liver and bile

DOG 16-6*	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
November 26...	34	36	29	99	10 8	9.6	9.8	30.2	trace	30.8	
November 27....				102				27.6	trace	30.8	Stools clay colored; no stercobilin.
November 29 ...	35	25	29	99	7.9	8.6	8.5	25 0	trace	31.0	
November 30.	35	26	27	88	10 1	10 5	9.5	30.1	0	30 8	
December 1 ..	30	31	33	94	12 2	8.9	9.0	30.1	0	30.3	Hemoglobin 108 per cent; beef heart diet.
Average .				96				28 6			

* Bile fistula operation September 2, 1915. Death, intoxication December 6, 1915. Usual mixed diet plus 200 grams boiled sheep liver with morning meal and 40 cc. fresh dog bile mixed with morning meal.

develop in consequence of long standing bile fistulas are of great interest but cannot be discussed at this time.

Autopsy in general is negative. Kidneys and other organs are normal. Liver is practically normal; no increase in fat. There is slight increase in the brownish color of the liver cells. The bile passages are all clean and pale throughout, even at the lower part of the fistula in contact with the drainage tube when it is in place. The common duct and the hepatic ducts are slightly dilated and thickened. Site of section of common duct is obliterated by dense scar tissue. The duodenal papilla is normal, and contains only mucus and no bile. Bile is completely excluded from the intestine.

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Table III (Dog 15–22) shows the initial loss of weight following a bile fistula from 30.8 pounds to 24.5 pounds due to the fact that the dog was quite fat when operated upon. The bile flow varies from 66 cc. to 97 cc. per six hours (average 76 cc.), and the bile pigments from 20 mgm. to 32 mgm. per six hours (average 25.5 mgm.), but the maxima and the minima for bile flow and bile pigments do not coincide in any way. Bile pigments are constantly present in small amounts in the urine. The usual hourly variations in secretion are noted. Sterco-

TABLE III

Normal dog—mixed diet and liver

DOG 15-22*	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
									mgm.	*pounds*	
April 21	33	30	34	97	9.6	9.5	7.8	26.9	0.25	27.3	April 20, R.B.C. 5,952,000: Hb. 94 per cent: W.B.C. 8,000.
April 22	25	24	26	75	7.1	9.1	11.9	28.1	+	27.5	
April 23	25	26	26	77	8.8	11.1	11.9	31.8	+	27.0	Stools clay colored; no stercobilin.
April 26	27	21	23	71	7.2	8.0	10.1	25.3	0.3	25.5	R.B.C. 5,824,000; Hb. 96 per cent.
April 27	15	26	25	66	3.6	8.3	7.9	19.8	0.2	25.0	
April 28	21	22	15	68	6.5	7.4	7.0	20.9	0.3	24.5	
Average				76				25.5			

* Bile fistula operation April 2, 1915, weight 30.8 pounds. Usual mixed diet plus 100 grams boiled sheep liver with morning meal.

bilin is absent, and bile presumably absent from the intestine, which is to be contrasted with Table IV in the same dog.

Table IV (Dog 15–22) is of considerable interest because the dog's stools now contain some stercobilin. This period is about four months after Table III observations of April. In May it was noted that the dog had gained weight up to his original weight of 30.8 pounds, and was in unusual condition for a bile fistula dog on a liver and mixed diet. Stercobilin was found in the feces constantly from this time on, and we were forced to the conclusion that by means of a small fistulous tract (as observed previously in the dogs at autopsy) a small amount

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of bile escaped into the duodenum when the pressure in the common duct was elevated.

From great numbers of observations on this dog and others with no bile escaping into the duodenum at any time, we are convinced that the collections from this dog, 15–22, represent his total output during the periods of six hours, we know that such fistulous tracts as this dog must have are very small and tortuous, and permit of only small amount of bile escaping even under high pressure. His output (Table III), before this fistulous tract into the duodenum was established, is practi-

TABLE IV

Normal dog—mixed diet and liver—bile in intestine

DOG 15-22*	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs	5-6 hrs.	Total 6 hrs.			
										pounds	
August 17	18	16	18	52	10.2	7.9	8.6	26.7	0	33.3	Hemoglobin 129 per cent
August 18	14	11	18	43	9.1	9.0	9.7	27.8	0	33.3	
August 19	17	14	16	47	9.2	8.5	8.8	26.5	0	33.3	
August 20	16	17	15	48	9.4	7.8	8.6	25.8	0	33.3	Stools contain stercobilin
September 15	27	17	25	69	8.3	6.3	6.8	21.4	0	33.	
September 16	23	17	28	68	9.1	8.2	10.7	28.0	0	32.8	
September 17	18	20	26	64	8.1	9.7	11.1	28.9	0	33	R.B.C. 7,848,000: Hb. 125 per cent: W.B.C. 9,600
September 18				56				26.7	0	33	
Average				56				26.5			

* Bile fistula operation April 2, 1915. Usual mixed diet plus 200 grams cooked sheep liver with morning meal.

cally identical with that in Table IV, the after period. His output of bile compares exactly with that of similar dogs with complete fistulae, shows the same fluctuations and the same average output. When the dog is put up in harness with a tube draining his gall bladder, it seems almost certain that all the bile escapes through this tube as the flow takes place by gravity. When the dog is curled up in his cage with his bile fistula partially closed and compressed by his posture, much bile escapes into the cage, and a small amount escapes into the duodenum along the path of the resected and ligated common duct. This bile is sufficient to maintain the dog in normal condition.

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Table V (Dog 15–22) resembles in general Table IV, and shows the same constantly high hemoglobin curve. There is a little loss of weight, but the dog is in perfect condition. This table covers a period of five days *following* a period of four days of sexual intercourse and excitement, during which period of over excitement the bile output was double normal. We have more data on this point which we will report later. These data are given to explain in part at least the abnormally high initial curve of pigment excretion in this dog with the progressive fall during the week (Table V).

TABLE V

Normal dog—mixed diet

DOG 15-22*	BILE								URINE BILE PIGMENT TOTAL — SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
1915										*pounds*	
November 26....	13	18	19	50	12.5	15	14.5	42.0	0	31.0	November 11, hemoglobin 121 per cent.
November 27.....				39				36.8	0	31.3	Stools contain stercobilin
November 29.....	19	22	20	61	7.0	16.5	12.9	36.4	0	31.5	
November 30.....	23	23	23	69	6.2	7.4	8.8	22.4	trace	31.3	Hemoglobin 124 per cent
December 1.....	21	22	25	68	9.4	10.1	10.7	30.2	0	31.3	
Average.....				57				33.6			

* Bile fistula operation April 2, 1915. Mixed diet of meat, bones, bread, and no liver.

Table VI (Dog 16–5) is a good example of the fluctuation of the bile pigment curve which may be found following a bile fistula operation associated with icterus of a mild but definite degree. For some reason drainage was not good after the operation, and for many daysafter normal drainage was established we see the wide fluctuation in output from a minimum of 18 mgm. to a maximum of 58 mgm. The weight remains constant, and the dog is active and hungry. Any procedure taken up during any such period could give no information, and would only lead to confusion. We believe the icterus is in part responsible for these great fluctuations in bile pigment excretion. Gradually the curve of pigment excretion becomes more uniform as seen in the next Table VII.

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Table VII (Dog 16–5) again shows the cholagogue action of bile given with the food (compare Table VI), and gradually the bile pigment excretion becomes fairly uniform.

One notes on two days (October 1 and 5) that the second period of collection shows a very small excretion. This is not due to any error in collection, as the dog was under constant observation, and we are not prepared to give a satisfactory explanation. It has been noted

TABLE VI

Post-operative icterus—mixed diet and liver

DOG 16-5*	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
										pounds	
September 15.....	22	15	15	52	17.8	15.3	16.9	50.0	++++	30.5	R.B.C. 5,472,000: Hb. 80 per cent: W.B.C. 8,200: definite jaundice.
September 16.....	20	15	20	55	21.7	19.7	17.2	58.6	++++	31.0	Stools contain no stercobilin.
September 17.....	22	15	24	61	13.9	12.2	21.1	47.2	+++	31.3	
September 18....				47				34.9	++	31.5	
September 20.....	19	9	11	39	22.5	16.4	14.5	53.4	+	30.5	
September 21.....	24	8	21	53	12.7	4.2	14.2	31.1	+	29.5	Slight jaundice
September 22.....	13	8	14	35	4.3	4.0	9.5	17.8	+	29.5	
September 23.....	7	17	24	48	2.5	7.8	10.8	21.1	++	29.0	Stools contain no stercobilin.
September 24.....	20	17	33	70	3.1	5.0	11.9	20.0	++	29.0	
September 25.....				46				12.4	++	29.3	Slight jaundice.
September 27.....	14	21	19	54	7.0	10.6	9.2	26.8	++	29.8	
September 28.....	23	15	16	54	6.5	6.1	6.3	18.9	++	30.0	
Average.....				53				32.7			

* Bile fistula operation September 1, 1915. Usual mixed diet plus 200 grams boiled sheep liver with morning meal.

that soon after operation (two weeks) the introduction of the rubber tube may be followed by an hour or more of almost complete cessation of the bile flow. This may be due to a nervous reflex resulting from the slight pain caused by the catheter in the recent fistula or due to the excitement of the novel surroundings and an unfamiliar procedure. We may be able to give a satisfactory explanation as more observations accumulate, but at present we believe such periods of inhibition of flow may be due in part to some nervous reflex.

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TABLE VII

Slight icterus—mixed diet and fresh bile

DOG 16-5*	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
										pounds	
September 29.....	27	17	23	67	8.3	4.0	9.5	21.8	++	30.3	Stools contain no stercobilin.
September 30.....	15	24	20	59	6.2	5.5	7.4	19.1	++	30.5	
October 1.....	34	8	36	78	10.7	2.9	11.3	24.9	++	30.5	Slight jaundice
October 2.....				62				29.3	++	30.5	
October 4.....	27	16	20	63	9.1	6.5	8.8	24.4	++	30.8	
October 5.....	31	10	25	66	12.4	4.3	12.4	29.1	++	31.0	
October 6.....	26	21	25	72	9.8	10.3	10.1	30.2	++	30.8	October 22—R.B.C. 6,360,000: Hb. 93 per cent: W.B.C. 13,400.
Average.....				67				25.5			

* Bile fistula operation September 1, 1915. Mixed diet plus 30 cc. fresh dog bile with morning meal and 60 cc. fresh dog bile with evening meal.

TABLE VIII

Poor condition—duodenal ulcer

DOG 15-16*	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
									mgm.	*pounds*	
April 14.....	55	27	44	126	17.3	7.7	10.9	35.9	0	42	April 5, R.B.C. 5,776,000: Hb. 90 per cent: W.B.C. 7,200.
April 15.....	41	42	24	107	9.2	10.3	6.6	26.1	0	41.5	Stools contain no stercobilin.
April 16.....	35	53	19	107	8.5	15.5	6.8	30.8	0	41.0	
April 19.....	22	27	30	79	18	23.1	28.5	69.6	0.7	39.5	Slight jaundice.
April 20.....	25	27	23	75	14.6	16.9	19.0	50.5	0.5	39.3	R.B.C. 5,408,000: W.B.C. 7,000. Stools contain no stercobilin.
Average.....				99				42.6			

* Bile fistula operation March 24, 1915. Death April 28. Usual mixed diet plus 200 grams boiled sheep liver with morning meal.

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Table VIII (Dog 15–16) shows a good example of a dog which lost ground steadily after the operation in spite of careful feeding and every attention. His bile output is fairly constant, but the bile pigment secretion varies from 26 to 70 mgm. per six hours. He developed a slight grade of icterus and lost all appetite. He was sacrificed because of poor general condition.

Autopsy gave the following information. Liver seemed normal in gross, but under the microscope showed a little fatty degeneration. Bile passages all normal, and bile completely excluded from duodenum. Large duodenal ulcer (1.5 by 1 cm.) about 3 cm. below pylorus extending deep into muscular coats gives no evidence of hemorrhage. Prostate is huge. Microscope shows the common cyst adenoma of the gland. The dog was quite old. The old age and the duodenal ulcer may be in part responsible for the rapid failure of this dog after the bile fistula operation. However, he showed some signs of the characteristic intoxication which so frequently carries off the animals.

DISCUSSION

A study of the above tables will emphasize the point which Stadelmann insists upon with so much justice, namely, the normal flow of bile from a bile fistula dog is subject to wide fluctuations, which cannot at present be explained. This includes fluctuation in the total output and the bile pigment secretion, but it is to be noted that these maxima do not coincide. A low output of bile may contain a high total bile pigment content and vice versa. Stadelmann points out the fact that bile pigment and bile salt secretion have no relation, and he argues from this that the function of the liver cell is double and quite independent in these two respects. With this point in mind, it is obvious that one must be very careful in analysis of any observations on bile secretion. Experiments must be repeated again and again, and a seemingly unnecessary number of control observations must be recorded before and after the actual experiment. It is seen in the above tables that with care and proper food a dog may have a fairly long period of relatively uniform secretion. Such periods are most favorable for experimental work.

We realize that criticism may be offered against our six hour period of bile collection. Many of the recorded experiments of other workers show twenty-four hour collections or even periods of several days during which time the dog is suspended comfortably in a sling. Others

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use ten or twelve hour periods for several consecutive days. After some observations, it seemed best in the long run to make shorter daily collections over many weeks or months, obtaining the bile every day during the same hours, the dog having a constant daily routine. The dog can live a pretty normal existence, and, most important of all, can maintain a pretty constant bodily condition for a long period of time. Such bile collections perhaps represent more nearly the normal flow as it occurs in a normal dog under laboratory conditions.

It will be seen after a careful study of the above tables that these bile fistula dogs on the mixed diet have a pretty uniform *average* excretion of bile pigments—about 1 mgm. per pound body weight per six hours. Our animals are even more constant in this respect than those studied by Stadelmann and his co-workers, but our average is practically identical with their published reports. This indicates that the method used by Stadelmann (spectrophotometric) gives the same general results as our method. The figures given by Brugsch, Kawashima and Yoshimoto are about three times as high as those just reviewed, and can scarcely be accepted as correct.

SUMMARY

Our experiences with bile fistula dogs indicate that bile is essential for the life of the animal on a mixed diet of meat, bones, and bread. If bile is *wholly* excluded from the intestinal tract, the dog loses ground steadily, shows intestinal disorders accompanied by blood in the feces, and usually within a month dies with peculiar symptoms of intoxication.

Fresh pig's bile given by stomach tube and dried ox bile given in capsules will sometimes improve the condition but not to any notable degree.

Fresh dog's bile mixed with the food will sometimes give good results if the dog will eat the mixture. Given by stomach tube the results are not favorable.

Cooked liver added to a mixed diet usually keeps the dog in good healthy condition for a long period of time. At present we are not prepared to explain this observation, but the fact may have some clinical application.

Under very uniform conditions the bile pigment excretion may form a pretty uniform curve, and experimental variations under such circumstances will have some value. The usual average bile pigment excretion amounts to one miligram per pound body weight per six hours,

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but there are some individual variations and considerable daily and hourly variation.

When a dog is not in good condition and perhaps is suffering from icterus or cachexia or both, we may see very great fluctuations in the bile pigment excretion curve. Experimental observations under such conditions are worse than useless, and can lead to no conclusions of value.

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BILE PIGMENT METABOLISM

II. Bile Pigment Output Influenced by Diet

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The preceding paper shows the normal bile pigment secretion in dogs with permanent bile fistulae. The normal fluctuations are obvious, and must be taken into consideration in the analysis of any experiments. This paper gives observations which make it clear that the curve of bile pigment secretion can be depressed below normal by a meat diet, and can be raised much above normal by a diet rich in carbohydrates.

It seems that this observation must dispose of the commonly accepted belief concerning the origin of bile pigments; namely, that they can be formed only by the breaking down of red blood cells. Can one assume that a carbohydrate diet will cause the dissolution of a small army of red blood cells to explain the fact that the output of bile pigment may be almost doubled in a sharp transition from a meat diet to a diet rich in carbohydrates? This seems improbable to say the least.

Methods and operative procedures, care of animals and collection of bile have all been described in detail in the preceding paper. We can not give all our experimental data on this diet question, but there is complete agreement in the fundamental observation that a dog will show a low bile pigment output on a meat diet and a definite increase (sometimes 100 per cent increase) on a diet rich in carbohydrates. The two following experiments are given in considerable detail, because the two dogs were under observation for a long period of time, in perfect health, showed a constant hemoglobin curve, and only traces of bile pigment in the urine at times. The observations on a single large dose of carbohydrate can be multiplied indefinitely, but they confirm the more difficult prolonged diet period experiments extending over weeks.

The Tables A, B, and C show that sugar by mouth will cause an increase in bile pigment output in a dog on a meat diet. There is a slight decrease in total bile flow during this period.

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TABLE A

Cane sugar feeding increases bile pigment secretion

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS EIGHT HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						Lean beef diet plus 200 grams cooked liver
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.			
										pounds	
October 21	24	23	26		10.0	1.4	13.2		0	32.0	
October 22	20	13*	12	22	9.2	14.3*	17.7	17.4	0	31.5	R. B. C. 7: 640,000; Hb. 123 per cent: W. B. C. 8600.
October 23	24				13.7				0	31.8	Stools contain stercobilin.
October 25	22	22	23		10.9	9.7	10.9		trace	32.0	
October 26	17	18	16		8.0	9.3	7.9		0	31.8	

* Given 80 grams cane sugar in 200 cc. water by stomach tube at the beginning of the third hour.

TABLE B

Dextrose feeding increases bile pigment secretion

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS, EIGHT HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						Lean beef diet plus 200 grams cooked liver
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.			
										pounds	
June 7	24	25	22		4.3	4.5	5.3		trace	31.5	Stools contain stercobilin
June 8	19	15*	11	14	4.6	6.7*	7.6	9.1	trace	31.5	
June 9	11	11	12		4.5	5.2	7.6		trace	30.5	
June 10	23	24	26		4.1	4.8	4.7		trace	30.8	

* Given 200 grams dextrose in 400 cc. water by stomach tube at beginning of third hour.

TABLE C

Cane sugar feeding increases bile pigment secretion

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS, EIGHT HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						Lean beef diet plus 200 grams cooked liver
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.			
										pounds	
October 14	22	24	22		5.9	10.0	11.1		0	32.5	
October 15	28	14*	15	16	5.6	8.5*	14.2	20.9	0	33.0	
October 16	22				11.9				0	32.8	
October 18	31	26	29		10.3	8.0	8.5		0	32.0	Stools contain stercobilin
October 19	22	24	27		9.2	8.5	10.1		0	32.0	

* Given 60 grams cane sugar in 200 cc. water by stomach tube at the beginning of the third hour.

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Tables D and E show that dextrose given intravenously will also cause a rise in the bile pigment curve of excretion, one experiment on a mixed diet and the second on beef heart diet. These experiments

TABLE D

Dextrose transfusion increases bile pigment secretion

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.			Mixed diet plus 200 grams cooked liver
										pounds	
May 18.....	19	21	18		6.8	7.1	7.0		trace	30.0	May 15, R.B.C. 5,888,000 Hb. 93 per cent.
May 19.....	19	23*	34	27	3.9	6.2*	12.1	10.0	trace	30.5	
May 20.....	18	20	21		3.6	5.4	7.1		+	31.0	Stools contain stercocobilin. May 22, R.B.C. 5,696,000: Hb. 91 per cent.
May 21.....	24	24	24		5.4	7.0	6.9		trace	31.0	

* 50 grams dextrose in 1000 cc. 0.7 per cent salt solution given intravenously at beginning of third hour.

TABLE E

Dextrose transfusion increases bile pigment secretion

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	7-8 hrs.			Beef heart diet
										pounds	
December 13.....	32	23	27		7.2	6.7	8.5		0	31	December 11, hemoglobin 131 per cent.
December 14.....	33	32	23		8.0	9.4	8.6		0	31.5	Stools contain stercobilin.
December 15.....	31	29*	16	15	7.5	11.5*	10.5	8.1	0	31.8	
December 16.....	23	22	23		14.1	14.5	12.2		0	31.0	
December 17.....	26				12.7				0	30.5	

* 600 cc. 6 per cent dextrose given intravenously at the beginning of the third hour.

show little decrease in bile flow, especially in Table D, where the larger amount of fluid (1000 cc.) was given intravenously. Such variations, however, come within physiological limits, and no importance is to be attached to them.

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Table F shows in a convincing way that a meat diet gives a much lower output than a diet rich in carbohydrates. The meat diet period of six days shows an average output of 29.1 mgm. bile pigment, which is not very low, as can be seen in the after-period of eleven days with an average of 25.6 mgm. (Table H). This same dog at other times on a meat diet has gone as low as 16 mgm. bile pigment output average of six days.

TABLE F

Bile pigment secretion on lean meat compared with carbohydrate diet

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						Boiled lean meat diet
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
1916										*pounds*	
October 25	22	22	23	67	10.9	9.7	10.9	31.5	trace	32	October 22, R.B.C. 7,640,000 Hb. 123 per cent.
October 26	17	18	16	51	8.0	9.3	7.9	25.2	0	31.8	
October 27	20	18	19	67	6.1	9.8	8.1	24.0	trace	31.8	
October 28	18	17	21	56	9.0	9.6	12.0	30.6	trace	31.5	
October 29	20	21	26	67	9.5	11.3	11.1	31.9	trace	31.8	
October 30				56				31.5	+	31.0	Stools contain stercobilin.
Average				59				29.1			

Change from lean meat diet to bread, milk and bones

November 1	15	16	18	49	13.5	15.1	16.	44.6	trace	31.5	Stools contain stercobilin.
November 3	16	18	20	54	14.4	17.1	16.7	48.2	trace	31.8	October 25, Hb. 123 per cent.
November 4	17	23	20	60	16.0	19.1	16.3	51.4	trace	31.8	
November 5	15	20	19	54	13.5	13.5	13.3	40.3	trace	32.0	
November 6				45				41.5	trace	31.8	
Average				52				45.2			

The sharp transition to the diet of bread, milk and bones gives a great rise to an average of 45.2 mgm. bile pigment, an increase of over 50 per cent bile pigment elimination. There is a trifling decrease in average bile flow from 59 cc. to 52 cc.

Table G shows that a continuation of the bread, milk, and bone diet does not maintain the bile pigment output at the maximum of 45.2 mgm. of the previous week, but the average is 39.7 mgm. bile pigment. This same fact is noted in another dog (Table K), and there is

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a tendency for the high bile pigment curve of a carbohydrate diet to approach the mean curve of a mixed diet. Also there is the same tendency for the low bile pigment curve of a meat diet to approach the mean curve of a mixed diet. This applies particularly to dogs kept for several weeks on a meat diet or a carbohydrate diet. The meat diet dog may show periods of rise in bile pigment output close to the mean curve of a

TABLE G

Bile pigment secretion on carbohydrate diet and fat

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS, SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						Diet of bread, milk, and bones
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
										pounds	
November 8.....	17	20	24	61	11.9	13.6	12.7	38.2	0	31.3	
November 9.....	19	23	21	63	13.3	14.2	12.8	40.3	0	31.3	
November 10.....	23	21	25	72	8.0	9.2	12.4	29.6	trace	31.3	
November 11.....	28	26	25	79	11.1	11.4	10.5	33.0	trace	31.5	Hemoglobin 121 per cent
November 12.....	16	23	22	61	12.6	13.2	12.6	38.4	trace	31.3	
November 13.....				55				42.5	trace	31.3	
November 15.....	14	23	21	58	14.5	13.2	14.4	42.1	0	31.0	
November 16.....	18	23	20	61	15.0	18.2	12.6	45.8	trace	31.5	
November 17.....	20	19	23	62	16.7	15.8	15.2	47.7	trace	31.3	
Average.....				64				39.7			
Same diet plus 100 cc. cotton seed oil with morning and evening feeding											
November 18.....	13	18	19	50	15.2	16.6	16.2	48.0	0	31.5	
November 19.....	24	23	19	66	10.8	11.1	9.6	31.5	0	31.8	
November 20.....				65				27.8	0	31.5	November 30, hemoglobin 124 per cent.
Average.....				60				35.7			

mixed diet. The diet rich in carbohydrate may give a very high initial curve (perhaps double normal), which is apt to fall during succeeding weeks, but always remains somewhat above the mean curve.

Cotton seed oil fed with this bread, milk, bone diet is associated with a slight drop in bile pigment elimination, but another dog (Table K) gives negative results. We hope to do much more work with various fats and lipoids.

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Table H shows the after-period on a beef heart diet with an average output of 25.6 mgm. bile pigment. The flow of bile is somewhat increased on this diet from 60 cc. to 83 cc. per six hours.

The importance of these observations (Tables F, G, H) lies in part in the fact that this dog was under constant observation with daily collections of bile for a period of about eight weeks in perfect health with uniform hemoglobin curve. The deduction to be drawn from changes in diet under such uniform conditions will be of value, and the sequence of events is not to be lost sight of: (1) Meat diet and low

TABLE H

Bile pigment secretion on beef heart diet

DOG 15-22	BILE								URINE TOTAL BILE PIGMENTS SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
										pounds	
December 2	39	27	28	94	7.8	10.3	9.9	28.0	trace	32.0	November 30, hemoglobin 124 per cent.
December 3	25	26	28	79	8.3	8.2	8.1	24.6	0	32.2	
December 4				79				24.9	0	31.3	
December 6	24	21	22	67	9.5	9.9	7.7	27.1	0	31.0	
December 7	27	28	31	86	8.3	10.1	12.3	30.7	0	31.5	Stools contain stercobilin
December 8	34	33	24	91	5.4	10.4	8.3	24.1	0	31.8	
December 9	38	28	26	92	5.1	6.7	7.6	19.4	0	31.8	
December 10	33	35	24	92	7.3	10.9	8.5	26.7	0	31.8	
December 11				64				28.2	0	31.5	Hemoglobin 131 per cent
December 13	32	23	27	82	7.2	6.7	8.5	22.4	0	31.0	
December 14	33	32	23	88	8.0	9.4	8.6	26.0	0	31.5	Stools contain stercobilin.
Average				83				25.6			

bile pigment elimination: (2) bread, milk, bone diet and very high bile pigment curve: (3) second period of bread, milk, bone diet and constant high pigment curve; (4) same diet with oil shows slight fall in bile pigment curve: (5) end period of beef heart diet with low bile pigment elimination (see table M).

Table J confirms the observations in Table F, but the change here is not so striking. On a meat diet the dog put out 27.7 mgm. bile pigment, and on a bread, milk, bone diet eliminated 37.5 mgm. bile pigment. It is to be noted that this dog was losing weight during this

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period, but seemed in good health, and the hemoglobin curve was uniform.

Table K shows a slight fall during the second week of carbohydrate feeding, but it remains above the meat diet period, and shows no depression as the result of adding cotton seed oil to the same diet.

TABLE J

Bile pigment secretion on lean meat compared with carbohydrate diet

DOG 16-6	BILE								URINE TOTAL BILE PIGMENTS SIX HOURS	WEIGHT	REMARKS
	Amount in cubic centimeters				Bile pigments in milligrams						
	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.			
										pounds	
October 25	30	27	22	79	10.8	10.3	9.7	30.2	0	34.5	October22, R.B.C. 7,640,000; Hb. 105 per cent: W. B. C. 8,600.
October 26	26	25	27	78	7.6	7.7	8.9	24.2	0	34.0	
October 27	25	24	23	72	7.3	7.9	9.6	24.8	0	33.8	Stools contain no stercobilin.
October 28	21	20	21	62	10.0	8.3	10.6	28.9	trace	33.3	
October 29	25	23	16	64	10.1	10.4	9.4	29.9	0	33.0	
October 30				54				27.8	0	32.5	Hemoglobin 103 per cent
Average				68				27.7			
Change from lean meat diet to bread, milk and bones											
November 1	16	18	20	54	10.8	13.8	11.9	36.5	0	31.5	October 30, hemoglobin 103 per cent.
November 3	17	18	20	55	13.4	11.3	15.0	39.7	0	31.5	
November 4	22	20	22	64	12.9	13.2	13.1	39.2	0	31.0	
November 5				54				37.8	trace	30.8	
November 6				66				34.1	trace	30.8	Stool contain no stercobilin.
Average				59				37.5			

The after-period (Table L) is unsatisfactory because rather too short as a result of the death of the dog. She was apparently in perfect health December 4, and it is proper to include this reading in the table. The next day she refused food, and vomited once, but did not appear sick. On December 6 she died with peculiar symptoms of intoxication, and the autopsy abstract is given in the preceding paper (following Table II—Dog 16–6).

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TABLE K

Bile pigment secretion on carbohydrate diet and fat

DOG 16-6	BILE: Amount in cubic centimeters 1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	Bile pigments in milligrams 1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	URINE TOTAL BILE PIGMENTS SIX HOURS	WEIGHT	REMARKS
										pounds	Bread, milk and bone diet plus 200 grams boiled liver
November 15	23	31	29	83	9.3	10.3	10.3	29.9	trace	31.5	October 30, hemoglobin 103 per cent.
November 16	30	31	29	90	10.0	11.7	10.3	32.0	trace	32.0	
November 17	22	28	35	85	9.7	13.0	8.6	31.3	trace	32.3	Stools contain no stercobilin.
Average				86				31.1			
Same diet plus 100 cc. cotton seed oil with morning and evening feeding											
November 18	28	27	30	85	10.5	8.4	8.8	27.7	trace	32.5	
November 19	35	33	35	103	14.8	8.8	8.7	32.3	trace	32.5	
November 20				112				30.2	0	32.5	
November 22	18	28	34	80	11.9	12.4	12.1	36.4	trace	31.0	
November 23	29	22	34	85	10.3	10.0	9.8	30.1	trace	31.8	
November 24	35	23	31	89	11.0	12.2	10.5	33.7	trace	31.3	December 1, hemoglobin 108 per cent.
Average				92				31.7			

TABLE L

Bile pigment secretion on beef heart diet

DOG 16-6	BILE: Amount in cubic centimeters 1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	Bile pigments in millimeters 1-2 hrs.	3-4 hrs.	5-6 hrs.	Total 6 hrs.	URINE TOTAL BILE PIGMENTS SIX HOURS	WEIGHT	REMARKS
										pounds	Boiled beef heart diet plus 200 grams boiled liver
December 1	30	31	34	95	12.2	9.0	9.0	30.2	0	30.3	Hemoglobin 108 per cent
December 2	35	39	30	104	7.8	9.9	8.1	25.8	0	30.0	
December 3	36	35	31	102	7.3	7.8	6.9	22.0	0	30.8	
December 4				112				17.6	0	31.3	
Average				103				23.9			

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TABLE M

Average readings from above tables

DOG	LEAN MEAT DIET		BREAD, MILK, BONE		BREAD, MILK, BONE DIET PLUS OIL		BEEF HEART DIET		REMARKS
	Bile	Bile pigment	Bile	Bile pigment	Bile	Bile pigment	Bile pigment	Bile pigment	
	cc.		cc.		cc.		cc.		
Dog 15-22	59	29.1	52	45.2					
Average per 6 hours			64	39.7	60	35.7	83	25.6	Weight, 32 pounds
Dog 16-6	68	27.7	59	37.5					
Average per six hours			86	31.1	92	31.7	103	23.9	Weight, 31 pounds.

This table gives the average readings of bile and bile pigment output during the various diet periods. They are arranged in the same sequence in which the experiments were performed. For study of the details one must refer to the other tables which are of greater interest.

DISCUSSION

From the above tabulated experiments it is clear that a diet rich in carbohydrates or sugar by mouth or dextrose intravenously will increase the secretion of bile pigments. We believe the data are sufficient to establish this as a fact, but how may we explain this increase in bile pigments following the administration of a carbohydrate? There are numerous possibilities which must be tried out by various experimental procedures, and we will merely mention a few of them.

Sugar feeding at once suggests a storing of glycogen in the liver, and its deposition in the liver cell may accelerate the metabolic activity of the cell or stimulate it to produce greater amounts of bile pigments than under normal conditions.

We must recognize, too, that a meat diet tends to depress the bile pigment secretion below the usual level of a mixed diet. Any explanation suggested to explain the carbohydrate stimulus must also explain the protein diet depression of bile pigment output. It is possible that a meat diet in dogs represents a normal condition, and that the low curve of pigment excretion on the meat diet is the true normal excretion. The rise on a simple mixed diet of bread and meat may then be explained by the addition of the carbohydrate and the maximum output on the bread, milk, bone diet as due to a great increase in the

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carbohydrate portion of the diet. It will be of considerable interest to observe the bile pigment output on a pure carbohydrate diet. This is a difficult type of experiment for a bile fistula dog, but it may give results of considerable value when carried to a successful termination.

Here it may be mentioned that we have pretty good evidence that bile or blood feeding do not greatly increase the bile pigment output. We hope to report detailed work on this important point in the near future.

When it is suspected that the liver can form bile pigments out of various materials other than hemoglobin, which is so closely related chemically to bile pigment, one first thinks of substances rich in the pyrrhole nucleus. Bile pigment or blood feeding should give a great increase in bile pigment output, which is not the fact. One must go back further in the development of the body pigments and ask; where does the hemoglobin come from? A prompt answer is made that hemoglobin is formed in the red cells in the bone marrow. But it is at least possible that these cells may merely put the finishing touches on this complex substance, which may be built up in great measure in some other tissue. In other words, there may be a prehemoglobin substance manufactured somewhere in the body, perhaps in the liver, which may be fixed by the bone marrow cells, and appear as finished hemoglobin. If it can be established that the liver cells form any such substance, a long step will have been made toward the solution of this complex question of pigment metabolism.

SUMMARY

A large dose of sugar by mouth will give a constant reaction in a healthy dog with a bile fistula. It will cause a definite increase in bile pigment excretion over a period of several hours.

The same rise in the curve of bile pigment elimination follows intravenous injection of dextrose.

A mixed diet in a healthy bile fistula dog is associated with a fairly constant *mean* bile pigment elimination.

A change to a meat diet will give a depression of this average bile pigment elimination.

A change to a diet rich in carbohydrates will give a sharp rise in bile pigment output—often 30 to 100 per cent increase. Such modifications of bile pigment elimination may be carried on indefinitely with a healthy animal.

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We believe established the fact that carbohydrates stimulate the excretion of bile pigments in bile fistula dogs, but a convincing explanation of this phenomenon we can not bring forward at this time. More work is required.

It seems, howevre, that these facts must overthrow the long accepted theory that bile pigment is formed only as a result of the disintegration of red blood cells.

It is at least possible that the liver has some constructive ability in pigment formation which can be modified by diet. It is also possible that the liver may be concerned in building up other body pigments than bilirubin—for example, hemoglobin.

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THE INFLUENCE OF HYPOTENSIVE GLAND EXTRACTS ON VASOMOTOR IRRITABILITY

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The observations herein recorded were obtained in the course of an investigation of the relation of pancreas extracts to vasomotor conditions. The original aim of our work was to determine whether or not pancreas extracts contain a specific hormone which could account for the favorable results which one of us (B.) has obtained in the clinical use of such material in arteriosclerosis, particularly in angina pectoris (1). Though this problem was not satisfactorily solved, certain observations made during the study have seemed worthy of record.

The technique employed was essentially that described in other reports from this laboratory (2). Dogs, under ether anesthesia, were used in all cases as experimental animals. These were first given standard injections of epinephrin and nicotin—of the former 1 cc. of a 1:50,000 solution, and of the latter 1 cc. of a 1: 2,000–4,000 solution—and the vasomotor responses noted by means of a mercury manometer and float. These reactions served as an index, respectively, of the condition of the peripheral musculature and of the vasomotor centers—a conclusion warranted by the work of Elliott (3), and of Langley and Dickinson (4), on the points of action of epinephrin and nicotin.

Following this preliminary standardization, the animals were given intravenous injections of gland extracts, and, after the blood-pressure had returned to a constant level, the reactions were again determined.

Various gland preparations were employed—fresh saline and glycerine extracts of the pancreas of the animal on the table, similar extracts of the pancreas of other dogs, commercial pancreatic extracts, principally the *holadin* of Fairchild, and saline extracts of salivary glands. Having convinced ourselves that the commercial material gave results comparable in a qualitative way with those of the other pancreas extracts, holadin was used in most of the subsequent experiments. Quantitatively

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however, it proved distinctly more potent than equivalent doses of fresh saline extracts of the gland.

The tendency of the extracts of most glandular tissues, when introduced intravenously, to produce a fall in the arterial pressure has been amply demonstrated. Whether this behavior is to be attributed to the cholin content of certain of these extracts; or, in the case of the pancreas, to the action of a hormone antagonistic to epinephrin; or is to be regarded as a manifestation of protein sensitization; or finally, is due to other, still unrecognized, factors, does not concern us in this place. Suffice it to say that the characteristic depressor effect of these substances was obtained with all of the pancreas, and with some but not all of the salivary gland, preparations; and that only the data derived from those which gave such a hypotensive result are included in this report.

In addition to the fall in arterial pressure following the injections there was observed a primary, slight pressor, effect; a gradual return to the normal tension following the depression, except in the case of extremely large doses, which caused a permanent hypotension; a fairly constant direct relationship between the dose introduced and the pressure fall; an acceleration of the heart beat; and an increase in the respiratory rate which persists even after the pressure and pulse have returned to the normal.

The commercial pancreas extracts employed in this study were prepared by macerating carefully weighed amounts of the powder in distilled water for twenty minutes, after which the mixture was doubly filtered and diluted to the desired concentration. A dose of 1 cc. of a 1 : 50 saline solution of the extracts was found adequate to produce a well marked fall in blood-pressure (fig. I).

Small doses, it was noted, did not affect the standard reactions to epinephrin, tracings due to the injection of the latter, before and after the introduction of the extracts, being practically alike. With heavy pancreas doses, i.e., 1 cc. of a 1: 50 solution, on the contrary, epinephrin failed to produce as complete a response as before.

In figure II are presented comparative tracings showing the reactions to epinephrin and nicotin before and after holadin injections. Graphs 1 and 2 are the reactions to 1.0 cc. epinephrin. An average normal blood-pressure of 120 mm. was maintained during the five determinations. Before the introduction of the pancreas extract (graph 3), epinephrin caused a pressor effect amounting to 28 mm. (graph 1). The reaction to the same dose after the pancreas injection is seen in

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Fig. 1. Tracings showing the effect upon blood-pressure of various pancreas extracts introduced intravenously. (1) Dog 8—Reaction to 1.0 cc. holadin, 1 : 50. (2) Dog 5—Effect produced by 1.0 cc. pankreon, 1 : 50. (3) Dog 9—Reaction to 2.0 cc. pankreatin, 1 : 25. (4) Dog 10—Curve following introduction of 1.0 cc. glycerine extract of dogs' pancreas. Blood pressure from femoral artery. Time intervals represent two seconds.

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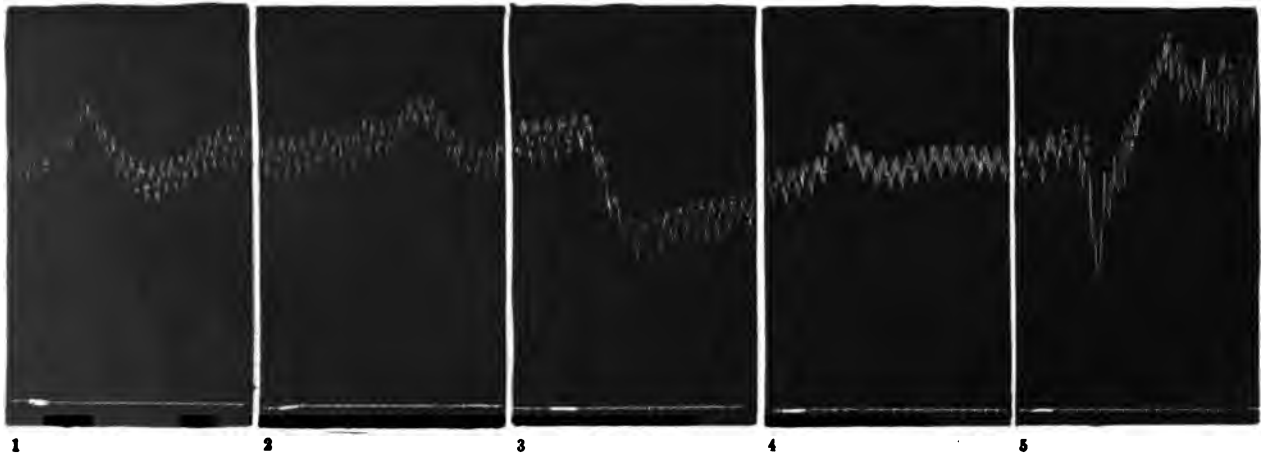


Fig. II. Tracings showing the effect of pancreas extracts upon the epinephrin and nicotin reaction. (1) Dog 3—Reaction to 1.0 cc. adrenalin, 1 : 50,000. (4) Tracing obtained from injection of 1.0 cc. nicotin, 1 : 2000. (1) and (4) are before introduction of 1.0 cc. holadin, 1 : 50. (3) Response to 1.0 cc. holadin 1 : 50. (2) Reaction to 1.0 cc. adrenalin. (5) Reaction to 1.0 cc. nicotin. (2) and (5) are after the holadin injection. Blood pressure taken from femoral artery. Time, 2 seconds.

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graph 2; the rise is 22 mm.—a loss of 6 mm., or more than 20 per cent as compared with graph 1.

Similar comparative tracings showing the behavior of nicotin are seen in graphs 4 and 5. In the former there is a pressor effect of 16 mm. and in the latter of 48 mm. The increased response to nicotin, in this case amounting to 300 per cent., is characteristic, though so pronounced a sensitization is not always observed. The pancreas injection caused a depression of 50 mm., the curve returning to the normal at the end of six minutes.

In view of the well-known tendency of many other gland extracts to cause hypotension upon intravenous injection, Ringer solution extracts of the submaxillary glands were employed in a small series of animals. The results were not constant either in respect to their hypotensive action, or as regards their augmenting the response to nicotin. Those extracts, however, which were most active in depressing the arterial pressure generally produced the most marked augmentation of the nicotin reaction.

We may summarize our work as follows: Extracts of the pancreas (glycerine, saline and commercial, and particularly the latter) when introduced intravenously into dogs, cause as a rule a pronounced fall in blood-pressure, associated with an acceleration of the pulse and respirations. Repeated injections of these extracts, in a dosage sufficiently large, produce a gradually diminishing response to standard epinephrin injections. A single dose of the preparations e.g., 1 cc. of a 1:50 solution, brings about a marked augmentation in the reaction to nicotin, which in many cases amounts to 300 per cent or more. Saline extracts of the submaxillary gland exhibit a similar, though not so constant, behavior.

As stated earlier in the paper considerable difference was noted in the depressor potency of various preparations used. A similar difference was observed in the augmentation of the nicotin reaction. This correlation suggests that the essential feature in the experiments is the hypotension. Possibly, the augmentation of sympathetic irritability is due to the cause postulated in another paper from this laboratory. i.e., a partial anemia of the medullary centers (5). The effect may be due, on the other hand, to action upon the sympathetic cells directly. The matter remains to be determined by subsequent research.

The reduction in the epinephrin reactions when larger doses were used indicates that a partial paralysis of the peripheral vascular musculature occurred. This conclusion follows, moreover, from the fact that low blood-pressure coincided with augmented vasomotor irritability.

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The peripheral vascular depression suggests that due to foreign protein poisoning or to anaphylactic shock. Dose for dose, however, our pancreas preparations were distinctly more potent than peptone solutions. The obvious difference from the anaphylactic reaction is that there was no reason to suppose that previous sensitization had occurred. Just what element, if any, is common to the three cases is an open question.

SUMMARY

Various pancreas and salivary gland preparations caused a vascular depression. This was associated with a decreased reaction to epinephrin, but with an augmented reaction to nicotin. Such extracts cause therefore, an augmented irritability of the vasoconstrictor centers.

We take pleasure in acknowledging our debt to Prof. R. G. Hoskins for his assistance in this research.

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THE ORIGIN OF THE ANTIBODIES OF THE LYMPH

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The origin of the antibodies of the body fluids—lysins, agglutinins, and opsonins have been the subject of very extended study. Most of these studies have been confined to blood only, but of late years the other fluids have come in for a share of the observation (1), (2), (3). In work published in earlier papers (2), (3), it was shown that if the six body fluids of experimental animals most readily accessible, were arranged in order of decreasing concentration, the following series would be formed: serum, thoracic lymph, neck lymph, pericardial fluid, aqueous humor, cerebrospinal fluid; but occasionally the order had to be reversed in the case of the last two. Clinical observations made with the Wassermann reaction show that in some cases the antibody against syphilis may be more concentrated in the cerebrospinal fluid than in the serum, at least, the cerebrospinal fluid shows a binding of complement when the serum does not. Such cases were never observed in our work on experimental animals.

With the observation of this apparently general rule of concentration of antibodies, it was apparent that two explanations of the presence of antibodies in the fluids of the body are possible; the antibodies may be formed in the blood, or reach the blood from extra vascular sources, and then pass into the lymphs and other body fluids; or, they may be formed in the lymph, or in some extra vascular cells and poured into the lymph and, then make their way into the blood stream, where the concentration becomes greatest by loss of water, by secretion, lymph formation, etc. If the first be the true condition, then in the passively immune animal, we should be able to trace the passage from blood to lymph with the true relation between concentrations as laid down by the rule; if the latter is the case, in the passively immune animal the passage from blood to lymph might be difficult and would not obey the rule laid down.

With these points in mind, the passage of antibodies from the serum into the body fluids of a normal dog, rendered passively immune by

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cross circulation with another dog highly immune to some antigen was undertaken. Our method of cross circulation consisted in placing a paraffined cannula in both central and peripheral ends of the cut carotid arteries of both animals. The central end of the carotid of the normal dog was then connected with the peripheral end of the carotid of the immune dog by means of a paraffined rubber tube filled with warm 0.9 per cent NaCl solution, and *vice versa*. All clamps were then removed and the peripheral end of the carotid in each case held in the fingers in order to be sure that the blood had not coagulated in the tubing. Although no anticoagulants were used, no particular difficulty with coagulation was experienced. Cross circulation was employed rather than bleeding the normal dog dry and refilling the vessels by transfusion from an immune dog, because, while the cross circulation method did not give as high a degree of passive immunity as the transfusion method would have given, it did not at any time alter the blood pressure conditions in the recipient and, therefore, it did not alter the physiological conditions under which lymph formation was taking place. Neither was plethora induced by over filling of the vessels. So far as we could determine, the passively immune dog was normal except for having yielded one-half of his own blood, to an immune dog, and having received an equal amount of immune blood in return.

The antigen used was commonly rat or goat blood or both. B. typhosus was used in several experiments. The methods used for determining the concentration of antibodies was that recommended by Hektoen (4) in an article to which the reader is referred for the details. In general all fluids were tested within thirty-six hours after withdrawal from the body, and in the interval they were kept in the ice-box. All fluids were heated at 49°C. for thirty minutes to kill complement. The hemolysins were reactivated with guinea pig complement. Fresh washed dog leucocytes from a pleural exudate induced by aleuronat were used in determining opsonins.

The results from our experiments were so concurrent that we feel that the publication of the protocol of a single experiment is sufficient, although 20 animals were used in the series.

Dog 19. Large brindle and white cur; weight 19 k.

May 21, 1910. Intravenous injection of 20 cc. 10 per cent washed rat corpuscle.
May 23, 1910. Intravenous injection of 5 cc. goat blood.
May 30, 1910. Anaesthetized with ether, trachael cannula inserted.
10.00 a.m. Samples of serum. Neck lymph and thoracic lymph collected.
10.09–10.19. Cross circulated from carotic with Dog 20.
10.20. Serum collected. Dog killed. See table 1.

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TABLE 1

Dog 19. This table shows the highest dilution at which the body fluids are just able to produce the reaction of the antibody under consideration in the body fluids of the immunized animal, and in the serum just after cross circulation with the normal. The number in each column represents the highest dilution at which the reaction in question occurred. Thus 768 = lysis in dilution of 1-768.

ANTIBODY	CORPUSCLE	10.00 A.M. (NORMAL)			10.20 (AFTER CROSS CIRCULATION)
		Serum	Neck lymph	Thoracic lymph	Serum
Hemagglutinins	Rat	768	96	192	96
	Goat	768	96	192	192
Hemolysins	Goat	96304	384	1536	6144
Hemopsonins	Rat	3072	768	1536	1536
	Goat	192	48	192	96

Dog 20. Large brown cur; weight 20 k.

10.00 a.m. Animal anaesthetized with ether. Tracheal cannula.
10.00. Samples of serum. Neck lymph and thoracic lymph collected.
10.09–10.19. Cross circulated from carotid with Dog 19.
11.20, 1.20, 3.20, 5.20, 7.20 } Sample of serum. Neck lymph and thoracic lymph taken.
7.25. Animal killed with ether. Lymphs were defibrinated and placed on ice until next day. See Table 2.

TABLE 2

Dog 20. This table shows the highest dilution at which the body fluids are just able to produce the reaction of the antibody under consideration in the body fluids of the normal dog, and in the body fluids of the same animal at various intervals after cross circulation. The number in each column represents the highest dilution in which the reaction in question took place. Thus 768 = lysis in a dilution of 1 in 768.

ANTIBODY	CORPUSCLE	SERUM							NECK LYMPH							THORACIC LYMPH						
Time		10.00	10.20	11.20	1.20	3.20	5.20	7.20	10.00	10.20	11.20	1.20	3.20	5.20	7.20	10.00	10.20	11.20	1.20	3.20	5.20	7.20
Hemagglutinins	Rat	24	768	768	768	768	768	768	12		96	96	96	96	96	12		192	192	192	192	192
	Goat	0	192	192	192	192	192	192	0		0	12	24	24	48	0		0	48	48	48	48
Hemolysins	Goat	192	3072	6144	12288	12288	12288	24576	96		96	384	768	1536	1536	96		768	3072	3072	3072	3072
Hemopsonins	Rat	12	6144	6144	6144	6144	6144	6144	12		48	192	384	1536	1536	12		1536	3072	3072	3072	3072
	Goat	0	192	192	192	192	192	192			0	6	24	24	24	0		24	96	96	96	96

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These results are graphically expressed in Charts 1, 2, and 3. The results show that if an animal is rendered passively immune by the introduction of immune blood under as nearly normal conditions physiologically as possible the concentration of antibodies of the lymph rises from the first. The rise is more rapid in the thoracic lymph than in the neck lymph, and the point ultimately reached is always higher in the former than in the latter, thus obeying the rule for actively immune animals. Hence, we believe that in measuring the antibody concentration in the lymphs of the passively immune, we are measuring what takes place in the actively immune animals.[1] The antibodies

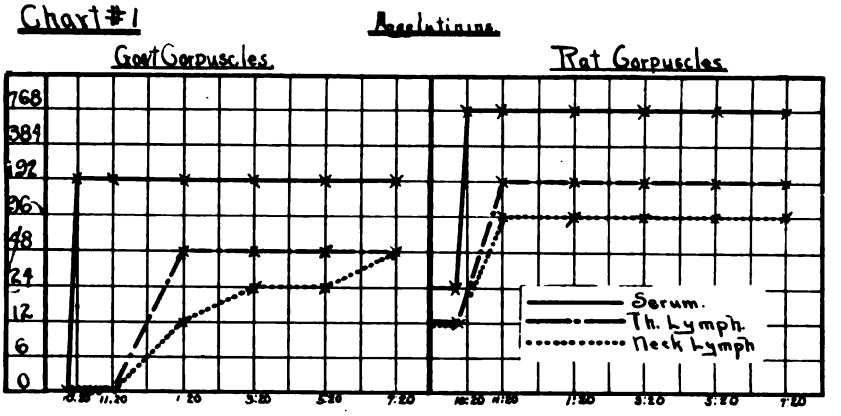


Chart 1. This chart shows the curve of the hemagglutinins in the body fluids of the normal dog after cross circulation with the immune.

reach the blood, and from that point make their way into the other body fluids by passage through the normal membranes until a certain equilibrium is reached.

CONCLUSIONS

1. The concentration of antibodies is greater in the serum than in the thoracic lymph, and greater in the thoracic lymph than in the neck lymph, not only in the actively immune animal but also in the passively immune animal; not only after equilibrium is established but at the time when active exchange is occurring.

[1] The method does not eliminate the possibility that some antibodies are added to the lymph from the tissues of the actively immune animal, nor do the authors see any method by which this phase may be studied.

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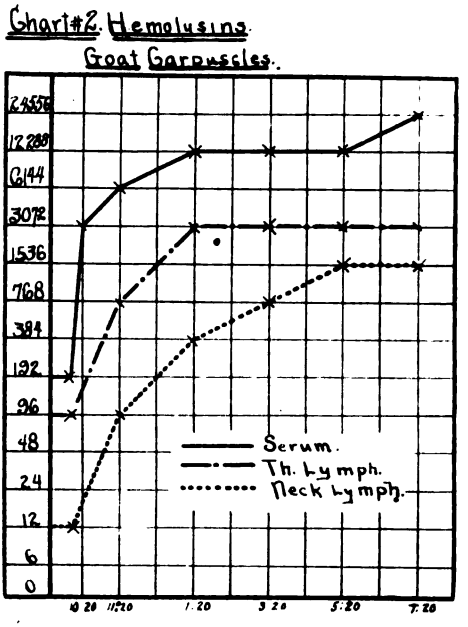


Chart 2. This chart shows the curve for the hemolysins in the body fluids of the normal dog after cross circulation with the immune.

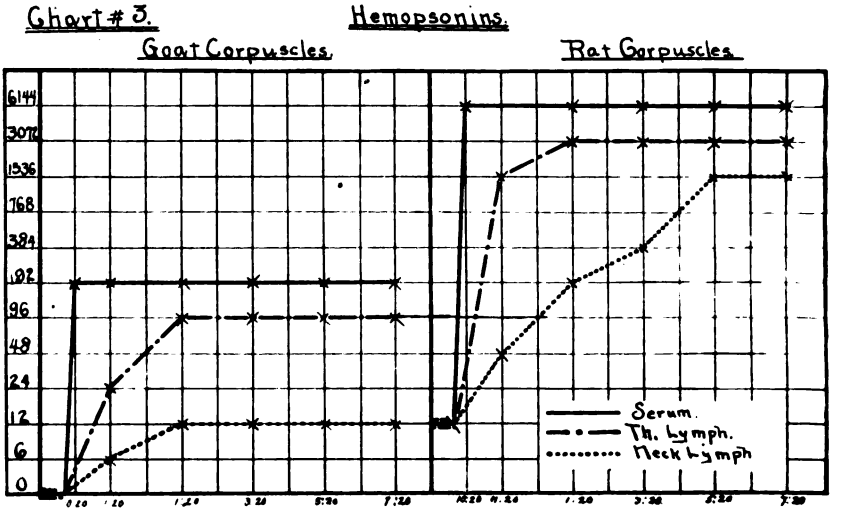


Chart 3. This chart shows the curve of the hemopsonins in the body fluids of the normal dog after cross circulation with the immune.

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2. The source of the antibodies of the lymph is the blood by direct exchange from that fluid. There is no evidence that antibodies originate from the tissues and are emptied into the lymph stream at the seat of formation.

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NOTES ON THE FALLING REFLEX OF CATS

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The ability of cats to turn in the air and to land squarely upon their four feet when falling is a very well known and interesting phenomenon. This response to falling is found invariably among normal cats; the rotation in the air occurs to either side dependent only upon the initial position in the air. Thus a cat with its legs inclining more to the right will turn to this side, making the subsequent rotation more easily. The reaction is so typical and inevitable that it seemed to offer an opportunity to test the hypothesis that many of the phenomena obtained in decerebrate animals were the result of an antigravitational tendency in the lower centers of the nervous system. This view-point was first advanced by Sherrington (7) who stated (p. 302) in regard to the rigidity following decerebration:

> The muscles it predominantly affects are those which in that attitude antagonize gravity. In standing, walking, running, the limbs would sink under the body's weight but for contraction of the extensors of the hip, knee, ankle, shoulder, elbow; the head would hang but for the retractors of the neck; the tail and jaw would drop but for their elevator muscles. These muscles counteract a force (gravity) that continually threatens to upset the natural posture. The force acts continuously and the muscles exhibit continued action, tonus.

The importance and significance of such an hypothesis seem to us to warrant further investigation. A search through the available literature failed to reveal any studies of the matter in animals which exhibited characteristic antigravitational reactions. Likewise no record could be obtained of any physiologic analysis of the factors involved in this typical rotation of cats when falling, although the physical aspects of this

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phenomenon have been more or less extensively investigated. In addition it was felt of value to ascertain the physiological anatomy of this response to falling—what pathways are involved, what anatomical structures yield the initial stimuli, and what influence the cerebral cortex has in the process.

The results of the experiments here reported have been, in the main, so consistent, that it seems pardonable to present them even though the series be small. All of the observations were made as acute experiments, allowing the cats to recover wholly from the anesthesia before testing the responses. These acute experiments were employed to avoid any possible assumption of function by other organs, to compensate for the experimental procedures. The animals were dropped varying distances upon a soft bed of straw in order that no discomfort could be caused by any failure of the normal rotation. For the routine observation the animals were held in a horizontal position, with their backs to the floor, and an effort was made to avoid imparting to the animal any rotatory impulse on releasing the hands.

A study was first made of this turning or falling reflex in normal cats. All of these animals invariably, when dropped, turned in the air so as to fall easily upon the feet. The turn in the air occurred almost immediately after the support was removed, the rotation being in the direction of the greater ease. All of these normal animals were able to complete the turn perfectly within a fall of 1 foot; some were able to accomplish the same twist of the body within the surprisingly short distance of 6 inches. When these normal animals were blinded with a tightly fitting mask, the rotation was made almost as perfectly and as quickly as by the normal animal. The landing upon the straw was, however, not as accurate in these masked animals, seemingly because of their ignorance as to the height of the fall.

The reflex rotation of these blinded cats in the air suggested immediately that the semicircular canals were the source of the initial impulse which occasioned the motor response. Hence, under full anesthesia, these canals were destroyed by an extra-cranial route—first on one side and subsequently upon the other. It was found possible to accomplish this destruction of the internal ear completely by this method without affecting neighboring structures.

If in a normal cat, the three semicircular canals on one side be destroyed, there will result a marked horizontal nystagmus to the opposite side, a partial rotation of the head away from the lesion, and a variable degree of ataxia (especially in the head). The animals strongly resist

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rotation away from the lesion, reclining always upon the operated side. Ewald (3), Cyon (2), von Stein (8) and others have described such acute symptoms for several other species. Somewhat similar acute disturbances were found by Lee (5) in the case of the dog-fish, after cutting the acoustic nerve—deflection of the eyeballs, change of position of the fins, curving of the body to the operated side and reclining upon this side.

When, however, one of these experimental cats with a unilateral lesion of its semicircular canals was dropped, it was always found, in this series, to rotate in the air, and to land upon the straw in a fairly normal fashion. The motor response in this rotation was never as perfect as in the normal cat, due possibly to the removal of some vestibular influence upon the muscular tonicity (Ewald). In addition, the animals usually required a greater distance in which to inaugurate their rotational movement.

Bilateral loss of the vestibular apparatus altered markedly the muscular, postural and ocular reactions of these cats. These animals exhibited no nystagmus of any character; the ataxia was frequently tremendously augmented; no preference was shown for either side on lying down. These changes in the reactions of the animal were particularly well shown in these observations when the experimental procedure was of two stages: in the first operation, the canals on one side were customarily destroyed with a resulting ataxia, nystagmus away from the lesion and a definite tendency to recline only on the operated side; after the loss of the second canal-system, the ocular and postural reactions disappeared with an accentuation of the ataxia.

Quite similar to our findings in the unilateral lesions were the reactions on falling shown by animals with both internal ears destroyed. Such cats, after complete recovery from the anesthesia, on being dropped, will turn in the air and land upon their feet. This result, at variance with our preconceived notions of the process, has been repeatedly found in every one of the animals observed.

But apart from the inevitable ataxia in the turning movement of the animals with one or both of their canalicular systems destroyed, there was found in these experiments a marked difference in the response of animals in which one internal ear or both internal ears had been destroyed. The animals with a unilateral loss of the semicircular canals were observed, on falling, to turn always away from the lesion. In other words, such an animal with the right internal ear destroyed, would inevitably twist in the air to the left, even though this necessitated a rotation of the body through 270 degrees. On the other hand,

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the destruction of both of the canal-systems permitted the animal to rotate in either direction (this being determined apparently by the angle to be traversed in the rotation).

Such findings inevitably force one to assume that other organs than the semicircular canals are able to initiate this falling reflex in cats. This other receptor which occasions the reflex seems to concern the eyes. If, as stated above, a normal cat be blinded, the falling reflex occurs as in the unblinded animal. But if these animals in which one or both of the semicircular systems have been destroyed, are blinded by the hood, no rotation will take place on falling. This finding was wholly constant in our series of animals in which both internal ears were destroyed; it was constant in a small majority of cases, in those animals in which only one canal-system was removed. The results, here, indicate that either the vestibular or optic systems may serve as the receptor organ for the rotation on falling; either of the two systems is sufficient to inaugurate the reflex, but if both are deprived of function, the turning reflex will not take place. The failure of blinded cats with one intact vestibular system to rotate is probably to be explained on the basis of a disturbed vestibular equilibrium; these animals are afraid or unable to trust, with one internal ear but recently destroyed, the impulses derived from the intact vestibule. Certain of these cats with unilateral loss of the semicircular apparatus will turn in the air when blinded; the rotation occurs, as in the unblinded animals, away from the lesion. It seems most likely that these animals learn more quickly than the others to place a certain reliance upon the stimuli coming from the intact canals.

Based on this evidence, the most logical interpretation seemed to be that both the eyes and the semicircular canals were able to give rise to impulses on falling that resulted in the rotation of the cat in the air. It is difficult to ascribe any greater importance to either of these organs; either seems wholly capable of initiating the same reaction. Probably both the ocular and vestibular mechanisms are normally employed in such a fall.

The physiological anatomy of the reflex on this basis seems fairly well established on its afferent side but not at all on its efferent pathway. Naturally one of the first questions to be solved here would concern the possible use of the pyramidal tract as the motor bundle. This was determined experimentally by testing the falling reaction of cats in which the motor cortex was ablated on one or both sides. Such a procedure would remove the connections of the pyramidal cells from

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their effectors, rendering it impossible for the animal to employ this cortico-spinal fasciculus. In these extirpations the motor cortex was often delimited by unipolar faradization; the ablations were always extensive, involving a considerable portion of the anterior half of the cerebral hemisphere. On recovery from the anesthesia, these animals rapidly acquired the power of locomotion and also of other purposeful movements—a finding to be expected in view of the observations of Brown and Sherrington (1) on the chimpanzee.

Cats with unilateral ablations of the motor cortex on dropping showed a wholly normal ability to rotate in the air and land upon their feet. That this reaction was not due to a bilateral control over the body-musculature exerted by the remaining motor cortex was demonstrated by the bilateral ablations. Most of these animals turned, on falling, with a very perfect adjustment to land upon their feet. A single animal in this series, with bilateral motor ablations, was able to turn only its front legs, the posterior part of the body not participating in the necessary rotation. This observation was of interest in view of the work of Marey (6) who, by means of photography, was able to demonstrate that the rotation of a cat in the air was a two-fold process—in the first phase the front legs were carried around and in the second, the posterior part of the body underwent the same rotation.

On being blinded, cats with this unilateral extirpation of the motor area were able, on falling, to twist in the air and to land as perfectly as the normal animal. But with a similar bilateral lesion, the blinded animals did not show such consistent reactions. Some of these animals were able to turn in the air on falling; others rotated only at times, falling the rest of the time without muscular reaction. Still others required tossing upon the straw in such a way as to introduce lateral motion into the spatial senses. The explanation of these varying results cannot be here given; it would appear that the extensive cortical lesion had interfered somewhat with the afferent pathways, necessitating for the rotational response stimuli from both ocular and vestibular mechanisms, in great strength. Thus, the added excitation, introduced by the lateral movements, might break through the thresholds and occasion the typical motor response.

The exclusion, by the observations just recorded, of a necessary participation of the pyramidal path in the motor response to falling, naturally led to further delimitation in this rather typical antigravitational response. The next observations were made on cats with both cerebral hemispheres entirely removed. These animals on recovery

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from ether were able to make progressive movements with both fore and hind legs, with no apparent excitation. Quite similarly coordinated movements could be obtained by appropriate stimulation. The optic nerves were undisturbed (reflex constriction of the pupil being elicited) as were also the semicircular canals. From these animals, however, no rotational reactions to falling could be elicited; the drops were not accompanied by any movements at all. This indicated that the cerebral hemispheres (consciousness?) were essential for the performance of this typical antigravitational rotation of cats.

Quite similar to these findings were the results of the observations made upon decerebrate cats. These preparations possess intact vestibular and auditory mechanisms (cf. Forbes and Sherrington (4)), with likewise intact rubro-spinal tracts. None of these decerebrate cats showed any tendency to turn in the air to avoid falling on their backs. And yet, these animals are able to make purposeful movements (as the scratch reflex) on proper excitation. As recorded previously by one of us (Weed (9)) destruction of the semicircular canals on both sides does not affect the existent rigidity. In these observations, the same failure to affect the rigidity was found on destruction of the canals on both sides; similarly, a previous bilateral destruction of the vestibular mechanism does not interfere in any way with the development of the typical decerebrate rigidity.

From these observations, then, a few conclusions may be drawn. The rotation of cats on falling seems to depend on excitations derived either from the eyes or from the semicircular canals. Loss of either of these organs of initial spatial relationship does not interfere with the falling reflex, but deprivation of both of these sensory fields abolishes this reflex. This rotational reaction also seems dependent upon some cortical influence (consciousness?) although it occurs after ablation of both motor areas. Decorticated (without cerebral hemispheres) and decerebrated animals do not, in our experience, show the slightest tendency toward rotation in the air. The rotation may be accomplished by the cat when deprived of the pyramidal tract by extirpation of the motor cortex.

Whether this rather typical reflex in cats, aroused by falling, may be considered an essential antigravitational reaction, would depend on many factors. The results here recorded surely offer no evidence either for or against the hypothesis that the muscular reactions in decerebrate rigidity are the result of an attempted resistance of gravity. The extensor thrusts of these animals, as pointed out by Sherrington

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(7), surely suggest an effort to overcome gravity. But such decerebrated cats as here described, show no tendency to protect themselves against falling by means of the normal rotational movements. Hence it may be assumed that the falling reflex is probably an acquired form of protective mechanism, dependent on influences from the semicircular canals and from the eyes, mediated largely, if not entirely, through the cerebral cortex.

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THE CIRCULATION OF THE BLOOD IN MAN AT HIGH ALTITUDES

III. The Effects of Physical Exertion on the Pulse Rate, Arterial, and Venous Pressures

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In our earlier studies of the circulation at a high altitude (1) we dealt with men who were comparatively inactive muscularly. In these men a marked increase in the rate of blood flow was found to occur with residence at the high altitude and this was associated in part with an augmented rate of the heart beat and a fall in the venous pressure, and in part with a dilatation of the arterioles. Kuhn (2) also has demonstrated by calculations made from determinations of the oxygen capacity of the blood, the total oxygen consumption, and the pulse rate, that the heart responds to the influence of lowered barometric pressure by increasing its output per minute.

In the present paper the immediate and after effects of various forms of exercise upon the arterial and venous pressures and on the heart rate will be considered. The data have in large part been collected in Colorado Springs and during two expeditions to the summit of Pike's Peak. Five subjects served in the first expedition, three of the men went up by railway train and were on the mountain fourteen days, June 16–29, 1914, while the other two walked up and remained five days. In the second expedition two men went up by train and remained three and a half days in October 1915. In as far as was possible the exercises used were the same at the two altitudes, 6000 and 14,109 feet.

The exercises considered were walks of fifteen minutes duration at the rates of three and four miles an hour, short rapid runs, work on a stationary bicycle, leg raising, and walking up Pike's Peak. The walks at three and four miles per hour were made indoors on smooth

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floors. It was impossible to secure comparable conditions at the two altitudes for the short runs therefore we had the men run as fast as they could in equal intervals of time.

THE PULSE RATE DURING REST

The early morning rate. Of all the circulatory changes due to diminished barometric pressure the acceleration of the heart rate is the most noticeable. In our recent expeditions to the summit of Pike's Peak we have continued the study of the early morning pulse rate in order to obtain a clearer understanding of the influence of muscular exertion and of mountain sickness on the heart. These counts were ma 'e on subjects in bed just after awakening. We have reported some of these data (1) and will now compare them with those of the recent expeditions. In our earlier report it was shown that the early morning pulse rate in men who were well and leading a sedentary life required several days after the ascent to reach its maximum rate. But in men who were mountain sick the rate was greatly augmented by the first morning and it thereafter retarded for several days. The data now presented cover observations on eight subjects, three of these men were studied during three and four trips and the others during a single sojourn. Five of the men have made the ascent on foot while one of these also went up twice by railway car.

For the men who ascended passively by train and were not mountain sick the rate, as found in our earlier work, was only slightly accelerated by the first morning and reached its maximum some days later. Sisco was healthy in two expeditions but had a slight bronchitis during a third. When well, in sojourn I, his heart rate on the first morning after the ascent had accelerated 4.5 per cent and by the fourth morning had advanced to 18.8 per cent; in sojourn III, by the first morning his pulse rate had increased 5.6 per cent and the maximum, 24.1 per cent, occurred on the third morning; while in expedition II when he was unwell, the pulse rate accelerated as much as 23.1 per cent by the first morning, the maximum, 28.8 per cent, occurred on the second morning. Schneider is always more or less mountain sick when he first goes to the summit of Pike's Peak. In expedition III, however, he was only slightly affected by the altitude, with the result that his pulse rate was up only 15.6 per cent the first morning after the ascent, the maximum rate, 21.9 per cent, was reached on the fourth morning. During the sojourns of more severe attacks of mountain sickness the

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maximum rate occurred on the first morning, thus in expedition I it reached 46.6, in II 42, and in IV 22.4 per cent. He was less ill in IV than in I and II. In the three expeditions in which Schneider was mountain sick the maxima exceeded the maximum for the trip when he remained comparatively well. Cheley, who was a member of expedition IV, suffered with a headache the first night and had his maximum acceleration, 22.4 per cent, the first morning. Havens ascended in expeditions I and III passively by train, but walked to the summit in II. Following passive ascent his pulse rate increased 7.1 and 7.4 per cent respectively by the first morning and reached the maximum, 21.4 and 18.5 per cent respectively, on the fifth day in each. Following the exertion of climbing the mountain his pulse rate reached its maximum acceleration of 26 per cent by the first morning.

European workers have generally found the increase in the heart rate on the first morning of a sojourn at altitudes above 14,000 feet greater than we have reported. Thus Durig and Kolmer (3) in their expedition to Monte Rosa obtained the following: Durig from an average at low altitudes of 61 had advanced to 80 or 31.1 per cent. Reichel from 64 to 92 or 43.8 per cent, Kolmer from 58 to 89 or 53.4 per cent, and Rainer from 56 to 97 or 74 per cent. A further acceleration occurred for from one to three days in three of the men. These data were obtained on men who had undergone considerable physical exertion in climbing the mountain. Our observations on men who walked up Pike's Peak indicate that the greater per cent of increase noted by Durig and Kolmer must be attributed to the influence of the fatigue of the climb. We found that men who climbed the mountain reacted very like those who became mountain sick.

Atwater, Eager, Gregg, Havens, and Munro climbed Pike's Peak on foot. Atwater and Havens were in better physical condition than the others. Atwater had prior to the trip been working regularly out of doors as a house painter. Havens trained for the two-mile run and was in excellent condition. Atwater's pulse rate had advanced the morning after the ascent from 47 to 78 or 66 per cent, Eager's from 66 to 90 or 36.4 per cent, Gregg's from 57 to 73 or 28.1 per cent, Havens from 54 to 68 or 26 per cent, and Munro from 44 to 72 or 63.6 per cent. Atwater, Eager, and Havens reached their maxima the first morning while Gregg and Munro each reached the maximum on the second morning. Gregg's greatest increase was 33.3 per cent and Munro's 90.9 per cent. The amount of acceleration in the heart rate of our subjects who underwent the exertion of climbing the mountain varied

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between 26 and 66 per cent on the first morning, while for Durig and Kolmer's subjects it ranged from 31 to 74 per cent. Contrast these figures with those of our subjects who ascended passively by train, and were not mountain sick, in which the pulse had only increased from 4.5 to 15.6 per cent on the first morning. On the other hand our subjects who were mountain sick showed on the first morning of residence accelerations varying betwen 22.4 and 46.6 per cent; these correspond more nearly with those of the group who underwent the physical exertion of the climb than with those who were well and ascended passively.

In not one of our subjects did the pulse rate during the stay at the high altitude wholly return to his Colorado Springs normal. Cheley's pulse rate made a remarkable return in his short stay on the Peak. His early morning pulse rate in Colorado Springs varied between 44 and 54 but was usually nearer the lower figure. On the third morning it was down to 50. Havens, Schneider, and Sisco remained two weeks on Pike's Peak in the summer of 1914. Havens' rate varied in Colorado Springs between 51 and 56. The thirteenth morning he had a rate of 56, lower by two beats than in any other sojourn on the Peak. Schneider's rate in Colorado Springs varied between 60 and 67, his lowest early morning count on the Peak was 72. Sisco with a low altitude rate varying between 50 and 57 was as low as 56 on only one morning, the tenth spent on the Peak. These indicate that in some men longer residence at the high altitude might restore the low altitude early morning rate.

The influence of posture on the frequency of the pulse. It is well known that the heart beat is influenced by posture. At low altitudes, and also at 6000 feet, when the body is changed from the reclining to the sitting and then to the standing position the beat will vary as follows: from 66 to 71 to 81 on the average.

It has been claimed that at high altitudes the variations in rate due to postural changes are greater than at low altitudes. Thus Zuntz and coworkers (4) found the frequency for one subject while reclining to be 80 and sitting 92, 106, and 108; another reclining 66, sitting 96, and standing 109; while a third subject gave reclining 84 and sitting 100. Fuchs (5) likewise emphasized the fact that the percentage of change in the three postures was much greater on Monte Rosa.

In a former paper (1) we showed that the early morning pulse rate, with the subject still in bed, and the rate throughout the day, when in the sitting posture, gave approximately the same percentage of increase on Pike's Peak as at lower altitudes. The heart was shown

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to take a higher daily tempo and in general to vary proportionately around the new level.

During our last expeditions to the summit of the Peak the conditions for the study of the daily average of the pulse rate of subjects in the sitting posture were not as good as in the first expedition, which was the basis for our earlier report. It should be noted that in our recent expeditions each subject had daily to undergo a considerable amount of physical exertion. In a rough way the curves for the mean rate of the pulse for the sitting posture follow those of the early morning. This parallel condition of the two curves is most marked during the early days of residence on the mountain. In Sisco, during the expedition of longer residence, the daily mean remained high after the fifth day while the early morning rate showed a decline. He showed a remarkable decline in the mean rate the last two days of his stay. Havens' sitting rate followed the morning rate more closely but shows greater variation than Sisco's. His mean sitting rate fell the last days more decidedly than the early morning rate. Schneider's daily mean for the sitting posture remained rather constant throughout the entire stay as did his early morning rate. The retardation after ten days of residence noted in Havens and Sisco is similar to that observed by Douglas, Haldane, Henderson, and Schneider (6) in three of their subjects at the end of two weeks of residence.

A comparison of the mean pulse rates for the sitting posture in the three groups, the well carried up passively, the mountain sick who also went up by train, and those fatigued by climbing the Peak, brings out the same differences noted for the early morning counts. For those who ascended by train and remained well the percentage of increase in the mean on the second day, or the day following the first night spent on the Peak, over the Colorado Springs average varied between 4 and 15 per cent. In the mountain sick group the acceleration ranged from 23 to 33 per cent. Among those who walked up the mountain the increase in the mean rate on the second day ranged between 18 and 44 per cent. Three of the men who walked up the Peak had on the second day a mean rate of 105, 101, and 100. These men showed a retardation each succeeding day. The other two showed the highest mean on the third day and this also was the day on which the early morning rate was greatest.

Our records do not show the range of variation throughout the day among those fatigued by the climb to be greater than for the men who ascended passively. Eager's heart rate ranged on the second day

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from 96 to 114, on the third from 84 to 110, on the fourth from 80 to 112, and on the fifth from 80 to 106. Munro gave the following: second day 81 to 106, third 94 to 108, fourth 80 to 96, and fifth 80 to 100. Others gave similar records. On contrasting these data with those tabulated by Schneider and Sisco for men who did not undergo great physical exertion we find the percentage range no greater. The maximum range of daily variation in the pulse rate among the men who climbed the mountain, during the five following days, was 40 per cent. The daily variation among the men of our former study ranged between 28 and 53 per cent which was not a greater variation than we found to occur in the same men at lower altitudes.

Havens, it will be recalled, ascended the mountain by railway train twice and walked up once. For the first three days following passive ascent the daily mean heart rate was 73, 82, 80 and 78, 79, 82 respectively, while for the three days after walking up it was 87, 96, and 86. The heart, therefore, takes at the high altitude a high tempo at once as a result of the fatigue of excessive exertion in climbing the mountain, but only gradually increases its rate when under the influence of reduced barometric pressure alone.

An exceptionally slow pulse rate was noted in Atwater the day after he made the climb to the summit. On that day his pulse varied between 72 and 84 with a mean of 78 which was only 18.2 per cent above his Colorado Springs average. The next day his heart was more irritable and advanced to a mean of 90 which was 36.3 per cent above his low altitude average.

Fuchs (5) in a series of observations upon himself found the acceleration of the pulse rate, in the standing posture as compared with that while reclining, varied at Erlangen between 8.6 and 11.7 per cent; while at Capanna Margherita on Monte Rosa the acceleration rose to 27.3 per cent. During our last stay on Pike's Peak the changes in the frequency of the pulse were determined in the morning before dressing and in the evening upon retiring for the three postures, reclining, sitting, and standing. Repeated counts were made extending over half minute and minute intervals and the particular position was kept for from three to ten minutes or until the pulse rate remained constant. Both subjects of this study were somewhat mountain sick the first night. Cheley, however, while awakening the next morning with a slight headache recovered completely after getting up. Schneider did not wholly recover until the following day.

Cheley's early morning changes were greater on Pike's Peak than in

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Colorado Springs. The first morning no difference could be obtained between sitting and standing, and the total acceleration following the change from the reclining to standing position was then 12 beats. The next two mornings the difference between the reclining and standing positions was 22 and 20 beats. For the same positions in Colorado Springs the difference was in general 14 beats. The increase for the two postures on Pike's Peak varied between 20 and 42 per cent and in Colorado Springs ranged between 19 and 33 per cent. For Schneider the early morning variations due to changes in posture were about the same at the two altitudes. The total difference in the number of beats for reclining and standing postures on Pike's Peak for the three mornings was 21, 19 and 15 respectively and in Colorado Springs ranged between 15 and 19. The percentage of change on Pike's Peak varied between 20 and 25 and in Colorado Springs between 25 and 29.

The evening counts reveal some additional facts. In Cheley the differences in the number of beats for the reclining and standing postures when on Pike's Peak were less than those found in the early morning. For the three days the evening differences were 15, 17, and 13, a variation of from 19 to 26 per cent. In Colorado Springs the evening variations ranged between 6 and 17 beats or between 13 and 31 per cent. Schneider's reaction to changes in posture in the evening on Pike's Peak ranged between 13 and 28 per cent and in Colorado Springs between 13 and 27 per cent. Altitude differences in the amount of variation in heart beats were, therefore, clearly lacking in Schneider, while the postural differences for Cheley were only greater in the morning.

From our data on the influence of posture upon pulse rate it is evident that the heart is not necessarily more irritable to changes in body position at high than at low altitudes. In general it may be said that the heart works at an increased tempo in all postures at the high altitude. The height of the new level of heart rate differs with individuals. Some men show only a few beats of acceleration over the lower altitude, while some show an increase of ten and more beats per minute. An occasional subject may have a lessened rate as was the case with Haldane of the English-American Pike's Peak Expedition (6).

THE INFLUENCE OF PHYSICAL EXERTION UPON THE PULSE RATE

In the past a considerable number of observations have been made on the influence of high altitudes upon the heart rate during physical exertion. We have attempted in our study by the use of graded exer-

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cises to analyze this influence somewhat more fully than previous workers. At very high altitudes all investigators have found that a more marked increase in the pulse rate occurs during work than with the same exertion at low altitudes. Veraguth (4) reported that in Zürich the ascent of a stairs of 50 steps increased his pulse 32.4 beats and in St. Moritz during the first ten days of residence 47.3 beats. Mosso (7) had several of his soldiers raise a pair of 5 K. dumb bells once every four seconds. One soldier raised them 121 times in Turin, as a result the pulse rate rose from 62 to 68 while on Monte Rosa after raising the dumb-bells 119 times the rate rose from 94 to 120. Kronecker (8), Zuntz and coworkers (4), and Durig (3) have studied such changes in greater detail. Stern (9) in 1913 made a still more exhaustive study. He corroborated the greater high altitude increase in frequency observed by others and noted that the curve describing the fall in the pulse rate after exertion always had a secondary ascent, while at low altitudes it was a smooth curve. Durig and Kolmer (3) were of the opinion that the amount of pulse rate increase could only partially be explained by the physical powers of the person under observation. The degree of acceleration was nevertheless influenced by training and adaptation. They also found that the after effects of exercise lasted a longer time in the mountain sick.

Just what height must be reached before altitude accentuates the exercise pulse rate has not been determined but Durig and Kolmer were convinced that at 6000 feet there was no noticeable influence. Our own experience in Colorado Springs definitely confirms this conclusion.

A. The after effects of muscular work on the pulse rate

Bowen (10) from a low altitude study of the changes in heart rate resulting from bicycling found that when work ceased there was a sudden and primary fall in the pulse rate, followed by a slower secondary fall, the two being frequently separated by a period of stationary rate—a plateau. Lowsley (11) reported that after short periods of exertion the pulse rate usually goes subnormal, but after fatiguing and exhausting exercises the pulse rate returns to normal more slowly and only rarely passes into the subnormal stage.

Walks at the rate of three miles an hour for fifteen minutes. The influence of the high altitude on the reaction of the heart to the very moderate and easy exercise of walking at the rate of three miles per hour for fifteen minutes was definite only during the first few days

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of residence. Cheley after walking at this rate in Colorado Springs had on the average a 16 per cent acceleration in the heart rate, furthermore the heart returned to its normal rate by a uniform fall in from two to five minutes. The first afternoon spent on Pike's Peak the same exercise increased the heart rate from 86 to 120 or 40 per cent; this was followed by a rapid primary fall and then by a slower secondary fall which carried it down to 90 in six minutes, where it remained for twenty-two minutes. After this it again slowly dropped and was back to normal within the hour. The next morning the same walk raised the pulse rate 42 per cent, from 72 to 102 beats. It retarded rapidly to 90 where it remained for ten minutes and then gradually went down to 76 where it remained for more than an hour. The third day the acceleration was from 75 to 108 or 44 per cent. The rate returned to 81 in six minutes and then went up to 84 where it remained ten minutes. afterward it went subnormal. On the fourth and last day this walk caused an increase in the heart rate of 34 per cent, but the return to normal was quickly made, occurring in seven minutes which was nearly as rapidly as in Colorado Springs.

During the 1914 expedition Schneider walked twice for fifteen minutes at the three-mile per hour rate. The first time was on the second day spent on the Peak. His acceleration was then from 90 to 120 or 33 per cent. The return was gradual but slow. Eleven days later a similar walk increased the pulse from 94 to 102 beats or 9 per cent and the return to normal was completed within eight minutes. Schneider's average increase in Colorado Springs for this walk is 15 per cent and the heart rate returns to normal in from three to seven minutes. In the 1915 expedition Schneider's reaction to this amount of work was followed more in detail. The first afternoon the walk accelerated his pulse rate from 82 to 132, or 60 per cent. The rate fell to 90 in six minutes where it remained for fourteen minutes, it next went down to 88 and was there over an hour later. The second day this amount of work increased the pulse rate from 102 to 132 or 29 per cent, it fell to 101 in ten minutes and then returned to 104 where it remained for thirty minutes. On the third day the acceleration was 30 per cent, but the heart rate returned to normal within fifteen minutes. On the fourth day the acceleration was only 20 per cent and the return to normal occurred within five minutes. The reaction on this fourth day was a marked improvement over that of the first day and practically what ordinarily occurred at the low altitude.

From this series of observations it is evident that the adaptive

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changes, which occur within the body during the first days of residence on Pike's Peak, favor the heart during the exertion of walking at the

TABLE I

Effects of three- and four-mile walks

DATE	PLACE	PULSE RATE			ARTERIAL PRESSURES IN MM. Hg.					
					Systolic			Diastolic		
		Before	After	Change	Before	After	Change	Before	After	Change
					Three-mile walks					
					(Cheley)					
Average..	Colo. Sp.	69	80	11	113	112	−1	88	88	0
Oct. 15...	Pike's P.	86	120	34	106	134	28	87	91	4
Oct. 16...	Pike's P.	72	102	30	112	128	14	88	89	1
Oct. 17...	Pike's P.	75	108	33	110	122	12	88	88	0
Oct. 18...	Pike's P.	76	102	26	124	132	8	88	88	0
					(Schneider)					
Average..	Colo. Sp.	71	82	11	114	119	5	88	88	0
Oct. 15...	Pike's P.	82	132	50	126	143	17	89	98	9
Oct. 16...	Pike's P.	102	132	30	126			90	94	4
Oct. 17...	Pike's P.	92	120	28	118	126	8	88	90	2
Oct. 18	Pike's P.	90	108	18	121	122	1	87	90	3
					Four-mile walks					
					(Cheley)					
Average..	Colo. Sp.	70	93	23	113	119	6	88	89	1
Oct. 15...	Pike's P.	83	144	61	106	150	44	88	95	7
Oct. 16...	Pike's P.	76	114	38	105	132	27	87	91	4
Oct. 17	Pike's P.	72	126	54	114	132	18	87	91	4
Oct. 18...	Pike's P.	72	126	54	119	142	23	88	92	4
					(Schneider)					
Average..	Colo. Sp.	71	100	29	114	120	6	88	90	2
Oct. 15...	Pike's P.	90	150	60	118	150	32	88	102	14
Oct. 16...	Pike's P.	90	150	60	125	140	15	89	94	5
Oct. 17...	Pike's P.	84	141	57	114	142	28	89	94	5
Oct. 18...	Pike's P.	82	132	50	116	142	26	87	96	7

rate of three miles per hour. The influence of low barometric pressure was manifest these first days by the greater acceleration in the heart

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rate, in the great extension in the time required for the rate to return to normal, and in the plateau which occurred in the curve describing the return to the normal rate. This plateau was practically always present. Following this exercise the secondary ascent in the curve noted by Stern (9) was present only twice, once for each subject.

Walks at the rate of four miles per hour for fifteen minutes. As would be expected, the after effects on the heart rate from walking for fifteen minutes at the rate of four miles per hour in Colorado Springs are more marked than when walking for the same length of time at three miles an hour. The amount of acceleration is greater, the return to normal is delayed, and a plateau in the downward curve is the rule rather than the exception. Our data for studies of this form of exercise at the two altitudes appear in part in Table I.

Cheley had in Colorado Springs after this rate of walking an average increase in the pulse rate of 33 per cent and returned to normal in from six to twenty-two minutes. On the first afternoon spent on Pike's Peak this walk sent the pulse from 83 to 144 beats per minute or 73.5 per cent; it slowed quickly to 114 and more slowly to 90 where it continued for five minutes; it then went down to 84 for two minutes and after this returned to 87 where it remained. On the second day with an acceleration of 50 per cent the retardation went through much the same course. Starting with 76 as the normal at 10.40 a.m. it was at 84 an hour and a half after work ceased. On the third day the rate increased from 72 to 126 or 75 per cent, then fell to 78 in twenty-five minutes. On the fourth day exactly the same increase from the same normal was obtained, but the heart rate was still at 81 when the work was interrupted thirty minutes after walking ceased. A plateau appeared each day and in three the secondary ascent noted by Stern also occurred. Cheley's reaction to this amount of exercise did not show marked improvement in the four days spent on the Peak.

Schneider was under observation after this form of exercise in the two expeditions, 1914 and 1915. In 1915 his average acceleration in Colorado Springs was 42 per cent and the return to normal occurred within from six to twenty minutes. In the four days spent on Pike's Peak there was only a little improvement in the way he reacted to this amount of exercise. The pulse rate increased 60 beats the first day and 50 on the last day. After each walk the acceleration was greater than 60 per cent and the return toward normal very tardy, in fact the heart seemed to take a higher tempo for several hours after each period of walking.

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In the 1914 expedition Schneider walked this rate of four miles per hour for fifteen minutes on nine different days within a period of two weeks (see Table II). His average acceleration in Colorado Springs was then 31 per cent and the return to normal occurred within from five to twelve minutes. On the first day spent at the higher altitude he had an acceleration of 60 per cent, and the heart did not return to 72, the rate previous to the exertion, but remained at 93. A headache came on with the exercise. On the second day the rate went from 90 to 144, 60 per cent, then dropped to 102 where it remained during a half hour period of observation. On the next day the pulse, starting at 86, accelerated 53 per cent, then retarded to 96 where it remained three minutes after which, apparently without cause, it went to 102 for four minutes and then gradually fell during the remainder of an hour. On the third day the amount of acceleration was less and never thereafter showed as great an increase in beats. On the fifth day even with an acceleration of 54 per cent the heart rate returned to normal within twenty-four minutes. From that day the acceleration was around 40 per cent and the time of return to normal was gradually lessened, thus on the fourteenth day the rate came back to normal in seventeen minutes. Schneider, therefore, showed a marked improvement toward the close of the stay on the Peak but always had a greater reaction than in Colorado Springs.

After the second day on the Peak, Havens was not clearly influenced by the altitude when walking on the level at the rate of four miles an hour. Not once was his percentage of acceleration greater than it occasionally was in Colorado Springs. However the first two days showed some delay in the time required for the pulse to return to normal. In Colorado Springs the normal rate was restored in from six to ten minutes. On the first day at the higher altitude the return was made in twenty minutes; on the second day the rate fell to 78 where it remained instead of decreasing to 72, the normal. Every day thereafter there was a complete return in from seven to fifteen minutes. It should be noted that Havens had trained during the spring months for the two-mile run in track athletics.

Sisco was less affected by the altitude than Schneider, but to a greater degree than Havens, when walking at the rate of four miles an hour. He had when in Colorado Springs accelerations varying between 9 and 45 per cent, with an average of 23 per cent; while the rate came back to normal within from seven to fifteen minutes. On the first day of residence on Pike's Peak his pulse rate had increased 59 per cent

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immediately after the walk and required thirty-five minutes to come back to normal. On the next day the increase was less but the return was not completed in the thirty minutes of observation. His average increase, after the first day, for the twelve days was 35 per cent, 12 per cent higher than in Colorado Springs. The time required for return to normal was variable, on some days it required twelve and on others as much as twenty-five minutes. Sisco made in the two weeks, therefore, a good return toward his low altitude reaction but even at the last there continued some altitude influence during this exercise.

Atwater and Gregg also served as subjects for the four-mile per hour walk during the four days following their climb to the summit of the Peak. In Colorado Springs each reacted less to this form of exercise than other subjects studied. Atwater's maximum acceleration in Colorado Springs was 13 per cent, with a return to normal within one or two minutes; while Gregg's maximum increase was 17 per cent, followed by a subnormal period with normal restored within five minutes. On the second day spent on Pike's Peak Atwater's pulse rose from 84 to 120 or 43 per cent, returning to 90 in three minutes and remaining there during an observation period of forty minutes. On the next day, when his heart was more irritable, the rate went from 90 to 150 beats per minute or 67 per cent, then quickly fell in two minutes to 84, which was subnormal, where it remained for five minutes and was back to normal eleven minutes after work ceased. On the fourth day the acceleration was 33 per cent and the return was made in nine minutes. In the five days he did not nearly recover his low altitude reaction but showed a marked improvement. Gregg, who was apparently the more affected by the climb, had on the second day an acceleration from 102 to 156 or 53 per cent. His heart rate failed to return to normal in eighteen minutes, but came down to 108 and remained there for several hours. There was some improvement during the next three days, in that the return to normal occurred within from seven to twenty-five minutes; but he did not, however, react as in Colorado Springs.

The influence of the fatigue due to the climb was not as marked on Atwater and Gregg during the walk at four miles per hour as was to be expected in view of the observations on the early morning rate and the daily mean pulse rate for the sitting posture. Nevertheless considering that both men were less influenced by this walk in Colorado Springs than our other subjects we are inclined to believe that the fatigue did make their hearts more irritable than altitude alone would

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have done. Physical fitness no doubt accounts in part for the differences in reaction seen among our subjects, but the factors are difficult to analyze.

The data gathered from our study of walks show clearly that at high altitudes, during the first days of residence, there is a greater heart reaction to a given exercise than after adaptive changes have set in. At first the amount of acceleration and the time required for return to normal rate are as a rule markedly increased. Each man studied showed a return toward the low altitude reaction, in Havens this was complete in two days, in others it was not complete at the end of two weeks residence at the higher altitude. The number of heart beats per minute, both before and after exercise, was for all subjects clearly above the low altitude rate. Following the four-mile per hour walks on Pike's Peak the plateau practically always appeared, but the secondary ascent only occasionally.

A more rapid walk.. An experiment was made on Cheley at both altitudes after a more rapid walk. In Colorado Springs he walked for fifteen minutes at the rate of five and one-half miles per hour. In consequence his pulse rate rose from 63 to 158 and returned to normal in twenty minutes. On the second day at the higher altitude he walked for ten minutes at five miles an hour, with the result that his pulse rate accelerated from 78 to 180. It then fell in four minutes to 108 and more gradually in the next thirty minutes to 100, where it remained for over an hour when observations were discontinued. Cheley felt very uncomfortable for some time after this walk and was not willing to repeat it later, while in Colorado Springs he was only breathless after the greater exertion.

The after effects of a short rapid run. A run of 175 yards up the "Cog" road track in from thirty to forty-five seconds gave a maximum of effort. One half of this distance was on the level, the remaining distance had in part approximately a 25 per cent grade. In order to do, in Colorado Springs, a comparable amount of work in an equal period of time it was necessary to run 260 yards through the corridors of one of the college buildings, including the ascent of two flights of stairs. As a result of the run in Colorado Springs the acceleration of the heart was between 38 and 90 beats per minute or to from 40 to 176 per cent, and the rate frequently returned to normal within fifteen minutes and never required as much as an hour.

It was difficult and exhausting to make the run on Pike's Peak. On the first day, although the runner went as fast as possible, the best

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time made was between 40 and 45 seconds. Furthermore each man felt like quitting before reaching the end. After several days had been spent on the Peak the effort was not so great and the distance could be covered within from 30 to 35 seconds. The time required for the rate to return to normal was so long that we found it necessary to leave this form of exercise until late afternoon when other experiments had been finished. After these runs the heart of each subject took and held for several hours a rate ten to twenty beats above normal. The data for these runs appear in Table III.

Havens made the run on Pike's Peak ten times, the heart rate increased to from 72 to 206 beats per minute, with an average of 121; the percentage acceleration varied between 86 and 219. For Schneider in six runs the rate increased between 72 and 162 beats, average 105, and the percentage change ranged between 75 and 208 per cent. Sisco ran seven times, the heart rate increased to from 108 to 174 beats per minute, with an average of 135 beats; his percentage increase varied from 125 to 223. Atwater rán once and had an increase of 204 beats or 283 per cent.

Lowsley (11) studied the increase in the heart rate after exertion for five forms of exercise, moderate, rapid, vigorous, fatiguing, and exhausting. They have been named in order of an ascending scale as to heart acceleration. The average increase was after moderate 26, after rapid 33.5, and after exhaustive exercises 54 beats per minute. The greatest acceleration noted by him was 80 beats per minute. He found that the heart rate returned to normal within about one-half an hour after moderate exercise and one hour after rapid exercise; while it required three and a half hours after exhaustive exertion. Our runs in Colorado Springs give about the same effects that Lowsley obtained after rapid exercises; while those on Pike's Peak accelerated the heart rate far more than the most exhaustive exercise studied by him and the slowness with which the heart rate returned to normal corresponded with that of his exhaustive exercises. The exhaustive exercises studied by Lowsley were ten and twenty-mile races. It is evident, therefore, that the heart is put to a very severe test at high altitudes by rapid vigorous exercise.

B. Changes in pulse rate during the period of work

We used a stationary bicycle for this series of observations. The devise was somewhat unsatisfactory in that we could not with exactness control the amount of resistance to be overcome. Our data do

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not, therefore, permit of exact comparisons from the standpoint of work done. In each experiment the subject drove the bicycle at the same rate for from fifteen to twenty minutes. The pulse rate was counted for ten second intervals. The data obtained in Colorado Springs in nearly every instance correspond with those of Bowen (10) and of Lowsley(11). There occurred at first a rapid primary rise in pulse rate when work began, lasting from one to four minutes, and then, either without a pause or following a plateau, a gradual secondary rise which apparently in some of the experiments would have gone higher had the work been longer continued. Most of our data for this work on the bicycle were obtained on Havens, Schneider, and Sisco. A single experiment at both altitudes was performed on Eager and Munro.

The observations on Pike's Peak, in the 1914 expedition, were made on the third, eighth, and eleventh days. The response of the heart at the high altitude was very different from that found to occur at the low altitude. In five of the nine experiments the maximum rate was reached within a half minute after work began. In the other four the maximum came in the primary rise within from one to five minutes. The maximum was always followed by a marked retardation which finally ended in a plateau. Occasionally a secondary rise followed toward the end of the work period but the rate rarely again approached the maximum of the primary rise. Havens and Sisco always reached their maxima within the first minute of work, Schneider's pulse rate increased more gradually and once reached the maximum as late as five minutes after work began. In the last experiment on Schneider the primary maximum was reached in one minute, four minutes later the slowest rate occurred and this was followed by a gradual secondary rise which, toward the end of the work period, carried the rate back to the maximum of the primary rise.

The experiments on Pike's Peak with Eager and Munro, who walked up, show the same general curve of acceleration. The primary rise in each lasted four minutes, after this there was some retardation. Eager's pulse rate went from 96 to 130 in four minutes, and then retarded for four minutes to 120, after which it varied between 120 and 124. Munro's heart accelerated in four minutes from 96 to 138, and later retarded and varied between 130 and 134.

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ARTERIAL PRESSURES

In an earlier paper (1) we presented data on the arterial pressures obtained from men leading a comparatively inactive life. In the majority of men studied the high altitude did not influence the arterial pressures, yet in some it caused a slight fall, and in one man a marked rise.

In our later expeditions the daily routine demanded greater activity, each day the members of the party took part in several physical exercise experiments. It may be worth while, therefore, to compare control records taken at both altitudes just before the beginning of the physical work of the experiment. In every case the control determinations were made only after the subject had been sitting quietly for at least five minutes and had not been exercising within an hour, although he was permitted during this time to be busy with laboratory duties.

Atwater, Cheley, Gregg, and Havens had practically the same averages at both altitudes, as well as corresponding variations. During the first two days of each expedition to the high altitude Schneider had higher pressures than at the low altitude. After this period of high pressure his average was a few millimetres less than in Colorado Springs. He showed a disposition during the 1914 expedition, when the amount of exertion taken daily was greatest, to have a rise in the systolic pressure of from 4 to 6 mm. Hg during the latter part of the afternoon. This was only noticeable on the days in which the program of experiments was crowded.

Sisco was a member of three expeditions to Pike's Peak, in the spring and autumn of 1913, and in June 1914. Prior to the first two his Colorado Springs averages were: systolic 118 and 119 mm., diastolic 85 and 86 mm., pulse pressure 33 mm. During the corresponding sojourns on Pike's Peak the following averages were obtained: systolic 117 and 115 mm., diastolic 84 and 85 mm., pulse pressure 33 and 30 mm. Hg. While there was no change in the first there was a fall in the pressures in the second expedition. Prior to the 1914 expedition all of Sisco's pressures averaged higher than ever before, the systolic was 125, diastolic 89, and pulse pressure 36 mm. During the first two days spent on Pike's Peak his pressures averaged about the same, but thereafter throughout the remainder of the two weeks stay each was decidedly higher. The averages were: systolic 130, diastolic 95, and pulse 35 mm. Hg. Furthermore Sisco showed the same late afternoon rise that was noticed in Schneider. We were unable to account for the high

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pressures in Sisco. That altitude was the sole cause of the rise seems improbable because of the fact that his arterial pressures were, even before the ascent, above what we had ordinarily found in Colorado Springs.

All arterial pressure determinations for the expeditions prior to 1915 were made with an Erlanger sphygmomanometer and those in 1915 with a Tycos sphygmomanometer.

A. The changes in arterial pressure after exercise.

Ordinarily at the close of a work period the arterial pressure is up but it at once begins to decrease. Bowen (10) finds that the fall in the pressure is not so rapid as the primary fall of the pulse rate which it accompanies and that a subnormal period follows. Lowsley (11) studied the systolic, diastolic, and pulse pressures after work and finds that these invariably fall below normal and remain in this subnormal condition for a considerable period. The systolic falls more rapidly than the diastolic pressure.

The after effects of walking at three miles per hour for fifteen minutes. Cheley reacted to this walk in Colorado Springs with a slight fall of from 1 to 3 per cent in the systolic pressure, while the diastolic pressure remained unchanged. In several instances the return to normal was observed and was found to occur within from three to seven minutes. During the four days spent on Pike's Peak he had after the walk a marked rise in the arterial pressures but progressively decreased the amount of reaction with each day of residence. During the first afternoon the systolic pressure rose 26.4 per cent and remained up thirty minutes, the diastolic went up 4.6 per cent and returned to normal within three minutes. On the second day the systolic increased only 14.3 per cent and returned to normal, without going through a subnormal period, in ten minutes; while the diastolic pressure only went up 1.1 per cent and returned in two minutes. On the third day the systolic pressure increased 10.9 per cent. The return, which was less rapid than on the second day, required eighteen minutes. On the fourth day, as on the third, the systolic alone increased and this time only 6.4 per cent, it then quickly went to subnormal. The pulse pressure following these walks in Colorado Springs was as a rule lowered, while on the Peak it went up to from 8 to 24 mm. While Cheley showed a marked improvement at the higher altitude nevertheless the reaction was, even on the last day, decidedly above any obtained with the same exercise in Colorado Springs.

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The experiments upon Schneider while on the Peak gave irregularities. In Colorado Springs this walk raised his systolic pressure from 1 to 7 per cent and the diastolic either not at all or not more then 1 per cent. The return to normal was rapid, and occasionally there occurred a subnormal period of short duration. Following the walk on the first afternoon spent on Pike's Peak, in October 1915, his systolic pressure was up 13.4 per cent and the diastolic 10.1 per cent. Afterward the systolic went subnormal but was back to normal within ten minutes, while the diastolic returned to normal in six minutes. On the following days the systolic pressure did not clearly rise more than it frequently did in Colorado Springs, but the diastolic rose from to 2.3 to 4.4 per cent.

Arterial pressure changes after walking at four miles per hour for fifteen minutes. In October 1915 both the systolic and diastolic pressures were determined, while in 1914 only the systolic pressure was recorded. Since the 1915 data cover both they will be discussed first. See Table I.

When in Colorado Springs this walk raised Cheley's systolic pressure as much as 3.5 to 7 per cent and the diastolic pressure 0 to 2.3 per cent. After work ceased the fall in pressure always carried the systolic pressure into a subnormal period for from 3 to 10 minutes, after which it returned to normal. During the first day spent on Pike's Peak the walk raised the systolic pressure 41.5 per cent and the diastolic 8 per cent, both returned to normal within fifteen minutes but did not become subnormal. On the second day the increase was somewhat less, systolic 25.7 per cent, and diastolic 4.6 per cent. The diastolic returned to normal in four minutes, the systolic pressure was still above normal twenty-five minutes after work ceased. On the third day the systolic rose only 15.8 per cent and returned to normal within ten minutes. While on the fourth or last day it rose 19.3 per cent, was subnormal fifteen minutes later and was not back in twenty-five minutes. A diastolic increase of 4.5 per cent occurred the last three days. With this as after the slower walk Cheley had the greatest reaction on the first day and considerably less on the following days. It will also be observed that with greater exertion the altitude influence became more pronounced.

The after effects of the four-mile per hour walk on Schneider's arterial pressures have been studied during two expeditions. In Colorado Springs this work raised his systolic pressure from 4 to 9 per cent, with an average increase of 6 per cent and the diastolic from 0 to 3

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per cent or an average of 2 per cent. Each pressure returned to normal within about five minutes, and practically always passed through a brief subnormal period. In 1915, when both the systolic and diastolic pressures were determined, Schneider reacted during the first afternoon spent on the Peak with a rise in systolic pressure of 27 per cent, and in diastolic of 15.9 per cent. Both pressures returned to normal within fifteen minutes. On the second day a systolic increase of only 12 per cent was obtained, but the normal, or control pressure, 125 mm. was abnormally high. After the walk ceased the pressure quickly went subnormal to 114 and did not return in thirty-five minutes. On the two following days both pressures rose a greater number of millimetres and each time the subnormal was more prolonged than in Colorado Springs. In the 1914 expedition when only the systolic pressure was determined Schneider also showed throughout the stay of two weeks a greater rise than in Colorado Springs. These data will be found in Table II. On the first day of this sojourn the systolic pressure rose from the high level of 128 mm. to 148 mm. of Hg or 16 per cent, the return to normal was slow and without a subnormal stage. The high pressure found before exercise was associated with oncoming mountain sickness. On the second day the reaction was 20 per cent. Thereafter the rise in systolic pressure was not so great; but it was, with one exception, always greater than followed the same exertion at the low altitude.

The pulse pressure changes after the walk of four miles an hour were greater on Pike's Peak for both Cheley and Schneider. Cheley's pulse pressure increased in Colorado Springs only from 2 to 8 mm. Hg. The first day on the Peak the walk raised it 37, the second day 23, the third day 14, and the fourth 19 mm. Schneider's increase in Colorado Springs was from 5 to 7 mm. For the four days spent on the Peak the rise was 18, 10, 23, and 17 mm. Hg respectively.

Systolic pressure determinations made on Havens and Sisco after the four-mile per hour walk show, on the whole, similar changes and also bring out the fact that all men do not react to the same degree when under the influence of lowered barometric pressure. Havens systolic rise in Colorado Springs varied from 0 to 7 per cent, with an average of 2 per cent, the return was accomplished quickly and rarely passed into a subnormal period. On Pike's Peak his increase ranged between 6 and 11 per cent. It was not more the first day than after long residence but his pressure invariably went subnormal for a time. During the first four days the return to normal required from 7 to

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TABLE II

Walks at four miles an hour

DATE	PLACE	PULSE RATE			SYSTOLIC PRESSURE IN MM. Hg			VENOUS PRESSURE IN CM. H_2O		
		Before	After	Change	Before	After	Change	Before	After	Change
					(Havens)					
Average..	Colo. Sp.	70	94	24	127	130	3	12.4	14.8	2.4
June 16...	Pike's P.	69	81	12	122	132	10	1.5	6.0	4 5
June 17...	Pike's P.	72	90	18	126	140	14	7.5	11.5	4 0
June 18...	Pike's P.	81	102	21	126	138	12	10.0	14.7	4.7
June 19...	Pike's P.	84	120	36	122	130	8	12.8	18.3	5.5
June 20...	Pike's P.	74	102	28	132	140	8			
June 22...	Pike's P.	84	120	36	132	146	14	10.6	12.6	2.0
June 24...	Pike's P.	88	102	14				7.9	11.0	3.1
June 26...	Pike's P.	90	108	18	121	134	13			
June 29...	Pike's P.	70	96	26	130	138	8			
					(Schneider)					
Average..	Colo. Sp.	80	105	25	117	122	5	16	17.1	1.1
June 16...	Pike's P.	75	120	45	128	148	20	18.6	25.3	6.7
June 17...	Pike's P.	90	144	54	122	146	24	11.4	14.1	2 7
June 18...	Pike's P.	86	132	46	118	134	16	20.7	17.4	−3.3
June 19...	Pike's P.	91	132	41	119	134	15	11.9	13.8	1.9
June 20...	Pike's P.	84	129	45	120	138	18	11.8	12.9	1.1
June 22...	Pike's P.	87	132	45						
June 23...	Pike's P.	90	126	36	118	124	5	15.1	16.1	1.1
June 26...	Pike's P.	90	126	36	118	136	18			
June 29...	Pike's P.	92	132	40						
					(Sisco)					
Average..	Colo. Sp.	71	87	16	128	133	5	10.1	11.0	0.9
June 16...	Pike's P.	87	138	51	122	138	16	14.2	14.5	0.3
June 17...	Pike's P.	75	102	27	122	134	12	6.0	15.1	9.1
June 18...	Pike's P.	96	120	24	130	143	13	−0.5	11.0	11.5
June 19...	Pike's P.	87	114	27	130	144	14	1.6	2.2	0.6
June 20...	Pike's P.	75	114	39	128	135	7	6.4	8.8	2 6
June 24...	Pike's P.	93	132	39	132	144	12	7.2	8.3	1 1
June 26...	Pike's P.	90	114	24	134	143	9	0.0	3.9	3 9
June 28...	Pike's P.	78	102	24	128	140	12	2.8	12.1	9.3

12 minutes, which was longer than in Colorado Springs. After these first days it returned to normal in from three to seven minutes, which was still slightly above the average time required in Colorado Springs.

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Again it should be noted that Havens was in excellent physical condition as a result of athletic training.

Sisco gave in Colorado Springs after the four-mile per hour walk an increase of from 3 to 9 per cent in the systolic pressure. During the first day on Pike's Peak the rise was 13 per cent and on the next three days was 10 per cent. Several times thereafter the rise was about the same as occurred at the lower altitude. Sisco's normal, however, was during this period almost continuously from 4 to 6 mm. above his Colorado Springs average. It is evident then that on the Peak after this walk his systolic pressure was uniformly higher than after the same exercise in Colorado Springs. For the first two days the return to normal was delayed from 50 to 100 per cent.

In order to determine whether the fatigue due to climbing the Peak would accentuate the altitude arterial pressure reaction we had Atwater and Gregg take the four-mile per hour walk on the four days following the ascent. Atwater's systolic pressure rose 20, 25, 20, and 8 per cent; Gregg's went up 15, 22, 34, and 9 per cent. The time required for the pressure to return to the normal was greatly prolonged in the first experiment and shorter thereafter. The reaction of these two men was not as great as was obtained by Cheley and Schneider but greater than for Havens and Sisco. The experiments on the last day, when the pressures rose only 8 and 9 per cent respectively, indicate that these men reacted well to the altitude influence. A similar improvement was noted in the reduced amount of acceleration in the heart rate. It is quite evident, therefore, that the fatigue of the climb did not materially alter the high altitude arterial pressure reaction for this amount of physical work.

Our arterial pressure studies following the walks show that the pressures are higher, after a given rate of walking, on the Peak than after the same amount of work at a lower altitude. On comparing the changes resulting from walking at the two rates, three and four miles an hour, we find that the greater the exertion the more pronounced is the influence of lowered barometric pressure. It is also evident that the altitude influence is most marked during the first days of residence on the Peak.

The after effects of a short rapid run on systolic pressure. Rapid runs of 260 yards with the ascent of several flights of stairs in Colorado Springs caused the systolic pressure to increase between 25 and 67 mm. Hg. The average rise was 31 mm., while the percentage of increase varied between 17 and 58. The pressure invariably went

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subnormal within from 9 to 15 minutes, and was normal again within twenty minutes. Lowsley found, after the 100-yard dash, that the average rise in the systolic pressure of nineteen men was 36 mm. A subnormal depression of from 15 to 25 mm. followed while the return to normal required as much as one hour and ten minutes. The data obtained on Pike's Peak appear in Table III.

Havens' reacted to the 175-yard run when on Pike's Peak with a rise in systolic pressure that ranged from 56 to 72 mm. Hg, an average of 67 mm. The percentage rise varied between 40 and 64. The later changes were followed for from 20 to 50 minutes and in no instance was the normal restored. His return to normal and into the subnormal period was very irregular. On the first day the pressure went up from 106 to 174 mm. and in 38 minutes had only fallen to 126. The next day it went into the subnormal phase within ten minutes. The fall into the subnormal phase varied between 9 and 36 minutes. Haven showed no definite improvement in reaction during a residence of tw weeks.

Schneider's systolic pressure, as the result of the run in Colorad Springs, rose from 25 to 30 mm., while on the Peak the rise varied fro 37 to 65, with an average of 52 mm. In Colorado Springs the depre sion into the subnormal period occurred within from 9 to 13 minute Following the first three runs on the Peak the pressure remained abo the normal during the periods of observation which were 35, 28, a 30 minutes respectively. For every run thereafter his pressure we into the subnormal period within from 11 to 15 minutes. This redu tion in the period of high pressure was Schneider's only improvem in the two weeks.

Sisco showed the same marked increase in the systolic pressure the high altitude that was obtained on Havens and Schneider. T increase varied between 47 and 82, with an average of 63 mm. O once did he go into the subnormal period during the time of obser tion and then this required twenty minutes. His pressure after w remained above normal as long as from twenty to thirty-five or m minutes. No improvement was noted during the two weeks.

The rise in blood pressure found after these short quick runs Pike's Peak is in general comparable to the increase in systolic pr obtained in exercises of maximum muscular effort. McCurdy the systolic pressure in Boston for twenty-three men duri of the maximum lift for each man, the weight lifted w to 249 kilos. The average rise in systolic pressure

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TABLE III

Short runs on Pike's Peak

DATE	PULSE RATE			SYSTOLIC PRESSURE IN MM. Hg		
	Before	After	Increase	Before	After	Increase
			(Havens)			
June 16	78	162	84	106	176	70
June 17	84	156	72	144	210	66
June 18	78	168	90	134	200	66
June 19	81	180	99	126	198	72
June 20	90	258	168	138	202	64
June 21	90	252	162	134	200	66
June 22	94	300	206	140	196	56
June 23	90	240	150			
June 25	90	180	90	122	194	72
June 28	81	168	87	123	194	71
Average	85	206	121	130	197	67
			(Schneider)			
June 17	96	168	72	120	158	38
June 18	78	240	162	127	176	49
June 19	88	180	92	124	186	62
June 21	96	216	120	124	186	62
June 25	87	180	93	127	192	65
June 28	90	180	90	127	164	37
Average	89	194	105	125	177	52
			(Sisco)			
June 16	72	192	120	122	182	60
June 17	84	192	108	122	170	48
June 18	90	240	150	138	185	47
June 19	78	252	174	144	226	82
June 20	96	216	120	128	204	76
June 21	100	252	152	140	200	60
June 25	68	192	124	128	200	72
Average	84	219	135	132	195	63

lowest increase was 49 and the highest, except for one very unusual case was 108 mm. Hg. As a result of our runs Havens' average increase was 67 mm., only two below McCurdy's average; while Sisco's was 63

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and Schneider's 52 mm. Our lowest rise was 37 and the highest 82 mm. McCurdy had seven tests in which the pressure was 200 mm. or above; while Havens and Sisco each on four occasions had pressure of 200 mm. or more. It appears then that the heart and blood vessels in these exercises were subjected to almost as great strain as occurs in the most severe test the heart must ever undergo.

Robison, who was acclimatized to the altitude of 14,109 ft. by a residence of five and one-half months, made this run with the result that his pressure rose from 104 to 158 mm., a rise of 54 mm. This exceeds the average in Colorado Springs and suggests that long residence at the high altitude does not much reduce this increase.

B. The changes in the arterial pressure accompanying exertion

The changes in arterial pressure during exercise have been considered at low altitudes by Bowen (10) and Lowsley (11). These found a rise in pressure when work began which reached a maximum in from five to twenty-five minutes. This followed after the primary rise in pulse rate. While the primary rise in pulse rate occurred in from one to three minutes, the pressure rise required four or more minutes. The diastolic pressure rose and either followed the systolic curve or occurred somewhat later.

We have studied the blood pressure changes during two forms of work. The systolic was determined for five subjects while riding for from fifteen to twenty minutes on the stationary bicycle, and the systolic and diastolic on two subjects for a period of six minutes during which they, while in the reclining position, alternately raised and lowered the legs. In each kind of work the rate of movement was the same at the two altitudes.

The study during work on the bicycle was of the systolic pressure alone. Experiments during the 1914 expedition were made on the third, eighth, and eleventh days. In Colorado Springs we obtained during the work period, curves similar to those reported by Bowen and by Lowsley.

Havens' systolic pressure rose to the maximum in practically the same time at both altitudes. In three typical experiments in Colorado Springs the pressure rose gradually to the maximum in 10, 11, and 12 minutes respectively. The maximum was reached on Pike's Peak in the first experiment in 11, in the second in 13, and in the third in 6 minutes. The rate of rise was more rapid during the earlier minutes

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of exercise on the Peak. His systolic pressure rose in three minutes in Colorado Springs 14, 16, and 20 mm.; and on Pike's Peak in the same time 22, 24, and 24 mm. respectively. Schneider's systolic pressure also rose more rapidly on the Peak than at the lower altitude. In Colorado Springs the maximum was reached in from 12 to 15 minutes. On Pike's Peak it rose to the maximum in five minutes during the first, in nine minutes in the second, and in three minutes in the third experiment. Sisco also reacted more quickly at the high altitude. His rise in Colorado Springs was made in from twelve to fifteen minutes. The first experiment on the Peak gave the maximum in eight, the second in four, and the third in three minutes.

Since the effort during work could not be exactly controlled a comparison of the total amount of rise in the systolic pressure obtained at the two altitudes will not give as reliable evidence on the influence of altitude as the data for the walks and for leg raising. Haven's rise in Colorado Springs varied between 22 and 34 mm., on the Peak between 26 and 30 mm. Schneider had, in each experiment in Colorado Springs, a rise of 23; on the Peak from 20 to 34 mm. Sisco's rise in Colorado Springs was around 18 mm. and on the Peak varied between 22 and 35 mm. Schneider and Sisco reacted with a slightly greater rise at the higher altitude, Havens with the same at both.

Single bicycle experiments at both altitudes on Eager and Munro show their rise in pressure during exercise at the high altitude to be more rapid. Eager's systolic pressure rose in the first four minutes 4 mm. in Colorado Springs and 10 mm. on the Peak. Munro's pressure rose 6 mm. in two minutes in Colorado Springs, and 16 mm. on the Peak.

The leg raising exercises were conducted on Cheley and Schneider in the 1915 expedition. Two experiments were made on each subject while on Pike's Peak. While the exercise was not continued long enough to obtain the maximum rise yet in each experiment both the systolic and diastolic pressures rose more rapidly than in Colorado Springs. In the two studies on Cheley when on the Peak the systolic pressure rose 24 and 11 mm. Hg in three minutes, in Colorado Springs in the same time it rose 7 and 8 mm. For Schneider the increase in three minutes on the Peak was 28 and 34 mm. Hg and in Colorado Springs from 22 to 24 mm. respectively. The total rise in systolic pressure on Pike's Peak for Cheley was 27 and 14 per cent in the two experiments and for two in Colorado Springs it was 8 and 10 per cent. Schneider's rise in systolic pressure in Colorado Springs ranged between 25 and 30 per

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cent; on the Peak the first experiment gave an increase of 33 per cent. and the second 39 per cent.

The diastolic which was determined during the leg raising exercise followed the systolic pressure. The total rise was greater on the Peak. For Cheley the diastolic increase in Colorado Springs was 1 and 2 per cent; in the first exercise on the Peak it was 4 per cent, and in the second 6 per cent. The diastolic rise obtained on Schneider in Colorado Springs varied between 0 and 2 per cent, on the Peak it was 9 and 8 per cent respectively. Both subjects had a greater pulse pressure during exercise on Pike's Peak but Cheley had a greater increase than Schneider.

The studies of the arterial pressure changes during exercise show clearly that there occurs a more rapid rise in the pressure at the high than at the low altitude. The rate of rise is not so rapid as that of the pulse rate, the pressure rise lagging somewhat behind the acceleration in pulse rate. The total amount of rise in the arterial pressure also is, in general, greater during work on Pike's Peak than in Colorado Springs. One of our subjects, Havens, did not have a greater increase in pressure at the high altitude, but as noted before he was in better physical condition because of participation in athletics than others studied. The greater rise noted during exertion accords with our data obtained from the study of the after effects of work.

The influence of low barometric pressure during and immediately after exertion reveals itself in a more marked rise in all the arterial pressures, and in the prolonged delay in return to normal. The effects are more pronounced during the first days of residence at the high altitude. The improvement resulting from the changes of acclimatization is only evident for very moderate exertion. The greater the exertion the more pronounced will be the altitude effects upon the rise in pressure and the delay in return to normal.

VENOUS PRESSURE

In our earlier report (1) it was stated that in five out of six men studied the venous pressure was as much as 25 to 87 per cent lower on Pike's Peak than in Colorado Springs. Havens and Sisco showed the fall in venous pressure in three trips to the Peak, while Schneider was not affected in four trips. Three additional men have now been examined and found to have a lessened venous pressure at the high altitude. The fall in pressure was for Atwater 40 per cent, Cheley 25 per cent, and Gregg 60 per cent.

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The effect of exercise upon the venous blood pressure

The changes in venous pressure while riding a stationary bicycle. That the venous pressure rises during exercise can be demonstrated by a casual observation of the superficial veins of the hands during exertion. Hooker (13) investigated the extent of this rise at a low altitude and noted the time required to reach the maximum pressure in men working on a stationary bicycle. In nine experiments the venous pressure in the superficial veins of the hand rose between 6 and 14 cm. of water, and reached the maximum in from two to forty-two minutes, in six out of nine tests this was reached in less than fifteen minutes.

Our own experiments made on five subjects while riding on the stationary bicycle were continued for fifteen and twenty minutes. As has been stated earlier we were unable to regulate with exactness the amount of work done on the bicycle, nevertheless the work done, which was approximately the same, produced marked differences in results at the high altitude. During this work in Colorado Springs, Havens gave a rise in venous pressure in four experiments of from 1.6 to 6 cm. of water; on Pike's Peak, also in four experiments, the rise ranged between 3.2 and 11 cm. The minimum and maximum were both somewhat higher on the Peak. Schneider's venous pressure during rest is scarcely affected on the Peak. During this form of exercise in Colorado Springs his venous pressure rose to from 4.9 to 6 cm. and on Pike's Peak in three experiments from 1.7 to 4.9 cm. He, therefore, reacted less rather than more. Sisco, whose venous pressure during inaction is uniformly low at the high altitude, had increases in venous pressure in Colorado Springs while working on the bicycle of from 4.4 to 7.4 cm.; while in three experiments on the Peak the rise was 8.4, 9.1, and 9.4 cm. respectively.

A single experiment on each Eager and Munro was made two days after they had walked up the mountain. Each man had a greater rise in pressure on the Peak than in Colorado Springs. Eager's rose in Colorado Springs from 13.1 to 16.7 cm. or 3.6 cm. in twenty minutes, and on the Peak from 3.6 to 11.8 cm. or 8.2 cm. In Colorado Springs Munro's venous pressure rose from 16.1 to 20.7 or 4.6 cm., while on the Peak it went up from 2.6 to 15.8 or 13.2 cm. of water in twenty minutes.

In four out of five subjects work on the bicycle caused, on the average, a greater increase in the venous blood pressure at the high altitude. The maximum pressure during work was not as high on Pike's Peak in any one of these subjects as in Colorado Springs. This is

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accounted for by the fact that each had in rest a lower venous pressure while on the Peak. We were unable to find any regular differences in the time taken to reach the maximum venous pressure during the period of work at the two altitudes.

The after effects of exertion on the venous blood pressure. The changes in venous pressure were studied on five men after walking for fifteen minutes at the rate of four miles an hour. In Colorado Springs the pressure almost invariably went subnormal for a period within from eight to twelve minutes after work ceased, but on Pike's Peak a subnormal period was observed only twice and both of these occurred in Havens. The time required to reestablish the normal pressure was not appreciably different at the two altitudes.

The rise in venous blood pressure noted immediately at the end of the walk merits a more detailed statement. These data appear in Table II. As a result of these walks in Colorado Springs Havens' venous pressure rose from 1.5 to 3.8 cm. During the first four days of residence on the Peak the following rises were observed 4.5, 4.0, 4.7, and 5.5 cm. of water. On the seventh and ninth days the rise was less, 2 and 3.1 cm. respectively. Schneider reacted in Colorado Springs, for this amount of work, with increases in venous pressure of from 0 to 5.3 cm. On the first afternoon on the Peak, in the early period of mountain sickness, this walk increased the pressure 6.7 cm. During the remainder of the stay the rise in pressure was not greater than occurred in Colorado Springs. Thus five experiments in a period of nine days gave the following increases: 2.7, 1.9, 1.1, and 1.0 cm. and one reaction in which there was a decrease in pressure of 3.3. cm. of water. Sisco's venous pressure rose only slightly as a result of this walk in Colorado Springs—0.3 to 1.7 cm. There was practically no reaction on the first afternoon on the Peak. During the following ten days six experiments on him gave respectively 9.1, 11.5, 0.6, 2.4 1.1, 3.9, and 9.3 cm. of water rises in venous pressure.

The venous pressure reaction after these walks was carefully studied on Atwater and Gregg after they had climbed the Peak but was neglected in Colorado Springs. If, however, it is kept in mind that the venous pressure seldomly increases more than 5 or 6 cm. in young men in Colorado Springs as the result of walking for fifteen minutes at four miles per hour the data obtained from them on the Peak is of interest. Both men made the walk four times while on the summit, the first on the second day of residence. Gregg reacted with the following rises: 4.4, 9.2, 10.2, and 7.2 cm.; all except the first were greater

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than would have occurred in Colorado Springs. Atwater reacted differently. As a result of the first walk on the second day his venous pressure fell 2.1 cm., on the third day 10.8 cm., on the fourth day 1.8 cm.; while on the fifth and last day for the first time it rose 1.4 cm. On the last day his pulse rate and arterial pressure also reacted more nearly as at the lower altitude.

The study of the after effects of this walk on the venous pressure has shown the same general reaction that occurred during the work on the stationary bicycle. Schneider's venous pressure in this form of exercise, with the exception of the first day, was not influenced by low barometric pressure. Gregg, Havens, and Sisco clearly reacted with a greater rise in pressure; while Atwater on the first days reacted with a fall in pressure during the exertion.

PULSE RATE AND BLOOD PRESSURE CHANGES AFTER WALKING UP PIKE'S PEAK

The heart beat, the arterial and venous blood pressures were determined on Atwater and Gregg at intervals during a period of seven hours after they arrived on the summit. Both on arrival felt well and cheerful after walking a distance of 9.4 miles with a rise of approximately 7700 feet in four hours and fifteen minutes.

The first determinations were made as quickly as possible after they arrived at 12.44 p.m. Our data appear in Table IV. In Colorado Springs Atwater had a systolic pressure of 140, diastolic pressure 90, and pulse pressure 50 mm. His venous pressure averaged 9 cm. of water, and his pulse rate under 70 beats per minute. Two minutes after arriving his pressures were: systolic 146, diastolic 94, and pulse pressure 52 mm. Hg, venous pressure 11 cm. of water, and pulse rate 114. Thus each pressure was only slightly above normal. Practically no change in the pressures occurred within the following twenty minutes, but the pulse rate had retarded from 114 to 102. From that time on the pressures declined, the arterial pressures did not fall equally, the diastolic lagging behind. As a result the pulse pressure fell most rapidly, to 34 within the first hour. An hour and a half later, or about two hours and fifteen minutes after he reached the summit, the pulse pressure had again returned to normal. The systolic and diastolic pressures both reached their minimum values at the same time, two and a quarter hours after the climb ceased. Both of these pressures were, generally speaking, back to normal two hours later. The entire time for the fall in these and the return to normal required for the

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systolic and diastolic pressures four hours and fifteen minutes and for pulse pressure two hours and fifteen minutes. These figures correspond with those obtained by Lowsley (11) in his study of exhaustive exercises in which the men at low altitudes ran races of ten to twenty miles. The average times required for his subjects to return to normal were for systolic slightly less than four hours (232 minutes), diastolic two and two-thirds hours (160 minutes), and pulse pressure two and a half hours (152 minutes). In his studies of moderate, rapid, vigorous, and fatiguing forms of exercise the time for return to normal for systolic pressure rarely approached two hours, for diastolic one hour, and for pulse pressure one and one-half hours. The time required by Atwater to return to normal clearly shows the exhaustive nature of the climb to the summit of Pike's Peak. The total fall in the pressures was not as great as often follows many forms of exertion. A fall in systolic arterial pressure was noted by Durig and Kolmer (3) in all four of their subjects shortly after the climb to the summit of Monte Rosa. Explanations offered were that in one subject it was due to heart fatigue and in two to skin irritation. Undoubtedly each man was in the subnormal phase, which invariably follows exertion, at the time the determination was made. They made only a single observation on each man. Atwater's venous pressure began to fall at once on arrival. This resulted in a negative pressure of 4.2 cm. of water at the end of three hours. His venous pressure was still negative the following morning but within the twenty-four hours again became positive.

The pulse rate gradually retarded until, four hours and fifteen minutes after the ascent, it had reached 84 beats per minute. This was the lowest count obtained for Atwater on that day. Lowsley found that his subjects' pulse rate after exhaustive exertion returned to normal in about three and one-half hours. The evening meal again accelerated Atwater's heart so that seven hours after the accent the rate was 93.

We started to take Gregg's pressure nine minutes after arrival on the summit. He felt slightly uncomfortable at the time and yet to our surprise he fainted immediately after the systolic pressure of 94 mm. had been determined. Previously by the rough procedure of raising and lowering the hand while a superficial vein was watched it had been noted that his venous pressure was negative. Immediately after Gregg fainted we placed him on a bed where he at once revived. He fainted at 12.54 p.m. and on recovery two minutes later his heart rate was 102 beats per minute. Four minutes later his systolic pres-

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sure was 94, diastolic pressure 78, and pulse pressure only 16 mm. Hg. He rested on the bed much of the afternoon. The pulse pressure gradually improved throughout the next hour, reaching at the end 35

TABLE IV

Effects of walking up Pike's Peak

TIME	PULSE RATE	ARTERIAL PRESSURE IN MM. Hg			VENOUS PRESSURE IN CM. H_2O	REMARKS
		Systolic	Diastolic	Pulse		
(Atwater)						
Average.....	64	138	90	48	7.8	Colo. Springs.
12.46 p.m.....	114	146	94	52		Pike's Peak, arrived at 12.44 p.m.
12.50 p.m.....	114	144	93	51	10.9	
1.15 p.m.....	102	131	94	37	8.7	
1.35 p.m.....	108	126	92	34		
2.05 p.m.....	93	135	92	43		
3.00 p.m.....	90	130	89	41	2.2	
4.00 p.m.....	90	132	90	42	−4.2	
5.00 p.m.....	84	140	97	43	−3.0	
7.45 p.m.....	93	140	105	35		
(Gregg)						
Average.....	71	115	84	31	11.5	Colo. Springs.
12.54 p.m.....		94			Negative	Pike's Peak, arrived at 12.44 p.m.
12.56 p.m.....	102					Just recovered from fainting.
1.00 p.m.....	108	94	78	16		
1.06 p.m.....	108	100	80	20		
1.12 p.m.....	108	100	79	21		
1.25 p.m.....	102	102	74	28		
1.45 p.m.....	102				Negative	
1.55 p.m.....	102	114	79	35		
3.05 p.m.....	108	116	79	37		
5.15 p.m.....	96	112	79	33	−1.6	
7.35 p.m.....	102	120	93	27		

mm. which was somewhat above normal, this being 31 mm. in Colorado Springs. This change in pulse pressure was wholly the result of an improvement in the systolic pressure which increased from 94 to 100

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mm. in six minutes, and then more gradually to 114 mm. Gregg's averages in Colorado Springs were: systolic 115, diastolic 84, pulse pressure 31 mm. Hg, and venous pressure 11.5 cm. of water. For at least four and a half hours after the ascent Gregg's diastolic pressure was subnormal. The return of the systolic pressure to normal, therefore, raised the pulse pressure a few millimeters above normal.

The venous pressure remained negative throughout the day and the pulse rate high; the lowest count, four and a half hours after he arrived, was 96.

One of Mosso's (7) soldiers, on Monte Rosa, fainted after lifting two five-kilogram dumb-bells 150 times. Mosso was able to study the respiratory changes in detail and also counted the pulse rate. He reported that the respiratory and cardiac functions were contemporaneously modified. Six minutes had passed after the cessation of work before his subject manifested any weakness in the functions of the heart and breathing apparatus. The heart rate had fallen from 136 to 120 beats per minute at the beginning of the faint. On recovery it was only 104 per minute. Gregg's heart rate after recovery increased from 102 to 108 per minute where it remained for about twenty minutes. The delay in the onset of the attack of fainting, as was observed by Mosso and by ourselves on Gregg, is not unusual. After severe muscular work the physical condition of the worker may become worse for a time.

Gregg ate a light supper which sent his systolic pressure to 120 and his diastolic pressure to 93 mm., resulting in a fall in pulse pressure. The result of the evening meal for both Atwater and Gregg was a marked fall in the pulse pressure, associated in each case with a rise in the diastolic pressure. The heart rate accelerated for both men.

Unfortunately, because all members of the expedition were more or less incapacitated by the altitude and fatigue, the circulatory changes in Eager, Havens, and Munro were not carefully followed after their walk up the Peak. They arrived at 4 p.m. Eager's pulse rate was then 136 and three hours later had retarded only to 115 beats per minute. Havens at 4.30 p.m. had a rate of 128, at 7 p.m. it was 96, and at 8.30 p.m. had retarded to 82 beats per minute.

It is clear from our observations on these men who walked up the mountain, as is shown by the long subnormal period of the arterial pressure and in the prolonged high rate of heart beat, that the exertion is very exhausting and puts a serious strain upon the heart.

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CONCLUSIONS

The normal circulatory conditions for the majority of men at an altitude of 14,109 feet are an increased rate of the heart beat, an unchanged or slightly lowered arterial pressure, and a lowered venous pressure. Physical exertion, as at a low altitude, affects all three, the pulse rate is further accelerated, the arterial and venous pressures are both raised, however, the change in each is substantially greater at the high altitude. Furthermore the stimulating influence of lowered barometric pressure is the more pronounced the more vigorous the exertion, that is to say, the curves representing the acceleration of the heart rate and the increase in pressures rise more rapidly than the curve that expresses the addition in rapidity or vigor of exertion. These facts clearly show that the heart and blood vessels, the arteries but not the veins, in that the venous pressure rarely goes above the low altitude average, undergo a greater strain during exertion at the high altitude than they experience for the same form of exercise at the low altitude. The irritability of the circulatory mechanism during and immediately following physical work is greatest throughout the first days spent on the heights; it decreases, particularly during moderate exertion, as the bodily changes of acclimatization progress. For persons in excellent physical condition and who have reacted well to the altitude, the changes of acclimatization will permit of moderate exertion without the lowered barometric pressure manifesting itself by the more pronounced acceleration of heart rate and in increased pressure. It seems probable that in vigorous work even those who are best adapted to the high altitude will continue to give a more pronounced reaction than would occur at a low altitude.

That the above is a fair statement of facts is evidenced by the following points taken from our experiments. The muscular action required for standing, as determined by the difference in the number of heart beats between the standing and reclining postures, did not even during the first days of residence at the high altitude very materially accentuate the heart rate acceleration. The three-mile per hour walks at the low altitude resulted in the following average changes for Cheley: pulse 11 beats, systolic 0 mm., and diastolic 0 mm. Hg; the first day spent on Pike's Peak the increase was pulse 34 beats, systolic 28 and diastolic 4 mm.; on the fourth day pulse 26 beats, systolic 8 and diastolic 0 mm. Schneider in eleven days fully returned to his low altitude reaction. The four-mile per hour walk gave for Cheley the fol-

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lowing reactions: average increase in Colorado Springs pulse 24 beats, systolic 6 and diastolic 1 mm. Hg; first day on Pike's Peak pulse 61 beats, systolic 44 and diastolic 7 mm.; fourth day pulse 54 beats, systolic 23 and diastolic 4 mm. Three men remained for two weeks on the summit but did not fully recover the low altitude reaction although they showed marked improvement. One man, however, who was in excellent physical condition had about the same increase in heart rate at the two altitudes. In two weeks residence three subjects showed no improvement in the amount of the reaction following the short quick runs. The acceleration of the pulse rate and the increase in systolic pressure were enormous and corresponded to the changes others have noted in the most exhaustive types of exercise at low altitudes.

That the heart undergoes greater strain during exercise at the high altitude is also indicated in the longer after period of high rate of beat and high pressures and also in the prolonged subnormal period of systolic pressure. This subnormal period after the short quick runs was as prolonged as Lowsley found after exhaustive exercises.

Our observations show clearly that physical exertion makes greater demands on the heart and blood vessels at very high than at low altitudes. They also indicate that during the first days of residence at the high altitude physical work should be reduced to a minimum. The writer has seen two persons faint just after arriving on the summit because they ran, to escape rain, from the train into the restaurant. The reason is quite evident. It is apparent that it would be an easy matter to seriously injure the heart during the early days of residence at the high altitude. For persons untrained and unaccustomed to physical work the climb to the summit of such a mountain as Pike's Peak must mean a serious strain for the heart. Ravenhill (14) cites two deaths from the cardiac type of mountain sickness of men who underwent considerable exertion in ascending a mountain in the Andes to an elevation of 15,400 feet. He found that men who did not remain quiet the first day or two at that altitude almost invariably became mountain sick. That the risk to the heart is less for men who are physically strong because of athletic training is indicated by the way Havens reacted to exercises at the high altitude.

It is to be expected that living at a high altitude, especially when much physical work is done, might increase the weight of the heart, for all muscular exertion tends to increase the weight of the heart and the result of work at the high altitude would accentuate the tendency.

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Strohl (15) compared the heart of the alpine snowbird (Lagopus mutus) which ranges from an altitude of 6700 to 10,000 feet with the moor snowbird (Lagopus lagopus) which is not found above 2000 feet and found that the average weight of the heart for each 1000 grams of body weight was for the Alpine bird 16.30 and for the Moor bird 11.08 per cent. The hypertrophy of the right was greater than that of the left ventricle. Heger and Meyer (16) studied the hearts of guinea pigs and rabbits at two altitudes, by weighing and with the orthodiagraph, and found the hearts of the rabbits at the high altitude larger. Stohl made one observation of considerable interest in which he found that the heart of a young Alpine snowbird one and one-half months old had the same proportions in weight as that of the Moor snowbird, which suggests that the differences ordinarily observed at the two altitudes are due to greater muscular activity at the high altitude.

There is nothing in our work to show why the right ventricle should hypertrophy more than the left. We noted, however, that Zuntz and Schumburg (17) found in their study of the influence of marching on the heart that the right ventricle was dilated on sixty-two occasions while the left was involved only thirty-one times.

SUMMARY

1. (a) By the first morning spent at the high altitude the pulse rate had accelerated in the group that ascended by train and remained well to from 4.5 to 15.6 per cent, in the mountain sick group to from 22.4 to 46.6 per cent, and for those who walked up to from 26 to 66 per cent. The mean pulse rate showed the same group differences, while the variations throughout the day were the same for each.

(b) The frequency of the heart beat due to changes in posture was not materially different at the two altitudes.

(c) The observations on the after effects of walks for fifteen minutes at the rate of three and four miles per hour and of a short rapid run show that physical exertion accelerated the heart rate more at the high than at a low altitude, and that the altitude influence was disproportionately increased as the amount of work was increased. For moderate exertion the effects were greater during the first days of residence. After a time the three-mile walk did not call forth the altitude acceleration, but it persisted after the four-mile walk even after two weeks of residence. The time required for the heart rate to return to normal was also prolonged during the first days but was reduced with acclimatization.

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The short rapid runs were exhausting at the high altitude, the heart was accelerated on an average of 120 beats per minute and the rate remained high for several hours thereafter.

(d) During work on a stationary bicycle at the high altitude the heart rate increased to the maximum in the primary rise; while at the low altitude the maximum came later, in the secondary rise.

2. (a) The three arterial pressures were higher after a given form of work at the high than at the low altitude. The influence of lowered barometric pressure was the more pronounced the more vigorous the exertion. The reaction was most conspicuous during the first days of residence, but while lessened by acclimatization it did not wholly disappear after a residence of two weeks. The average rise in the systolic pressure after a short quick run was 61 mm., while the average pressure for each of three men was 177, 195, and 197 mm. Hg. These high pressures were similar to those caused by a maximum lift of weights. The delay in the return to normal was as prolonged as after exhaustive exercises.

(b) During work the arterial pressures rose more rapidly at the high than at a low altitude, the rate of rise was not as rapid as that of the pulse rate. Ordinarily at the low altitude the arterial pressure reaches its maximum before the pulse rate, but at the high altitude the pulse rate first comes to the maximum.

3. Physical work caused in five out of seven subjects a greater rise in the venous pressure at the high than at the low altitude. The maximum, however, was on the average less than at the low altitude because of the fact that the normal venous pressure was lower on Pike's Peak.

4. The exertion of walking up the Peak is shown to be exhausting and to put a serious strain upon the heart.

The writer wishes to thank generous friends who contributed toward the expenses of the expeditions to Pike's Peak. He is also indebted to the young men who so kindly served as subjects for these observations.

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THE APPEARANCE OF SUGAR IN THE SECRETIONS OF THE DIGESTIVE TRACT FOLLOWING THE ADMINISTRATION OF PHLORHIZIN

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Since v. Mering discovered in 1886 that the administration of phlorhizin renders an animal glycosuric, many attempts have been made to learn the nature of the phlorhizin effect. The article on "Phlorhizin Glycosuria" by Graham Lusk which appeared in the "Ergebnisse der Physiologie, 1912" contains a complete review of the literature on this subject, hence, only a brief statement of previous work which bears on the present communication need be given here.

It is generally accepted that the glycosuria following phlorhizin administration is accompanied by a hypoglycaemia, and that this serves to differentiate it clearly from other forms of glycosuria, in which a state of hyperglycaemia exists. Because the injection of phlorhizin into the renal artery is immediately followed by a glycosuria, and since no reduction occurs in the amount of blood-sugar in phlorhizinized animals, in whom the renal arteries or urethers have been tied, most physiologists have concluded that phlorhizin exerts a specific action on the renal cells, producing what might be termed a "renal diabetes." There have been several hypotheses advanced to explain the mechanism of phlorhizin glycosuria. The "vehicle theory" proposed by Minkowski, assumes that phlorhizin, being a glucoside composed of a glucose molecule and a substance known as phloretin, is broken down by the kidney cell into its constituent parts of which the sugar escapes into the urine, whereas the phloretin recombines with sugar brought to the renal cells by the blood to reform phlorhizin which again breaks down: and so the process goes on till finally the phlorhizin is completely excreted from the body. Another theory supported by the experimental data of Levene, Biedel and Kolisch, Pavy and Lepine, is that the renal cells under the influence of phlorhizin bring about a change in the blood-sugar which makes it diffusible and enables it to pass through the renal epithelium into the urine.

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Underhill, using maximal phlorhizinized dogs, found that an increase in blood-sugar resulted when the kidneys of the dog or rabbit were thrown out of function. This hyperglycaemia is not as great as in the depancreated animal, yet it indicates a specific stimulation of sugar production by phlorhizin. Underhill, nevertheless, recognizes a specific renal action in phlorhizin (1).

The explanation accepted by most physiologists is that phlorhizin specifically affects the renal cells either to increase their permeability to blood-sugar or to excite them to secrete dextrose.

All the hypotheses of phlorhizin action assume a specific action of the drug on the kidney. There are some observations, however, which point to the possibility of its having a more general action. In 1894 Levene found that a reducing substance is present in the bile of phlorhizinized animals, whereas none is found in the normal bile. Brauer, using a different method to free the bile of pigments, was unable to confirm this finding. Ray, McDermott, and Lusk could not detect any sugar in the bile vomited by phlorhizinized animals. Recently, Woodyatt has repeated the examination of the bile as directed by both Levene and Brauer, and has succeeded in showing that if the bile is properly prepared it will ferment with yeast and yield characteristic dextrosazone crystals (2).

Cornevin believed that phlorhizin has a direct action on the mammary gland but this work has not been confirmed by subsequent workers. Delmare reported the presence of a reducing substance in the sweat of a phlorhizinized animal. This work has never been repeated. Aside from those above mentioned, no attempts have been made to discover whether or not phlorhizin directly affects secreting glands other than the kidney.

DISCUSSION OF RESULTS

In the present research I have investigated the reducing power of the pancreatic, gastric, and salivary secretions in normal and phlorhizinized dogs.

The pancreatic juice

The experiments on the pancreatic juice were performed on dogs which had been starved for two or three days before the operation. The juice was collected from a cannula inserted in the pancreatic duct, and the pancreas was stimulated by the intra-venous injection of secretin, prepared as directed by Bayliss and Starling. In a few cases in

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which the amount of juice obtained by the action of the secretin was small, pilocarpin was given, but it failed to excite secretion in all but one experiment.

Samples of blood for blood-sugar estimations were taken at frequent intervals before and after the injection of the secretin. The pancreatic

TABLE 1

The sugar content of the pancreatic juice of normal dogs to which phlorhizin had been given two hours before the experiment

NUMBER OF EXPERIMENT	DATE	NORMAL OR PHLORHIZINIZED ANIMAL	STARVATION PERIOD IN DAYS	CUBIC CENTIMETERS OF JUICE OBTAINED	NYLANDER'S TEST	BENEDICT'S TEST	PICRIC ACID REDUCTION TEST	PER CENT OF SUGAR IN THE PANCREATIC JUICE	PER CENT OF SUGAR IN THE BLOOD BEFORE THE INJECTION OF SECRETIN	PER CENT OF SUGAR IN THE BLOOD AFTER THE INJECTION OF SECRETIN
1	10/26	Phlorhizin	2	25	+	+	+			
2	10/28	Phlorhizin	3	10	+			0.07	0.076	0.075
3	11/1	Phlorhizin	2	7		+	+	0.09	0.12	0.122
4	11/3	Normal	2	12	0	0	0		0.125	0.120
5	11/6	Normal	2	10	0	0	0			
6	11/8	*Normal	2	6	0		0		0.105	0.105
8	11/11	†Phlorhizin	2	6		0	0		0.100	0.110
9	11/13	Phlorhizin	2	12		+	+	0.10	0.13	0.13
10	11/15	Phlorhizin	1			+?	+	0.05	0.127	0 13
11	11/16	Phlorhizin	2	8			+	0.04	0.08	0.08
12	11/16	Phlorhizin	2	12		+	+	0.066	0.08	0.08
14	11/24	Phlorhizin	2	3				0.085‡	0 085	0.10
17	12 8	§Phlorhizin	2	2			+	0.035	0.085	0.10
19	12, 9	Normal	2			0	0		0.095	0.095
20	12, 10	Normal	0	12		+	+	0.06	0 175	0 18

* Pilocarpin was given after secretin.

† Urine gave marked reduction.

‡ Peculiar color reading questionable.

§ Phlorhizin injected intravenously after taking sample of normal juice which gave no reduction.

juice, obtained from normal dogs and from dogs to which two gram doses of phlorhizin dissolved in 1.2 per cent sodium carbonate solution had been given subcutaneously two hours earlier, was tested for its reducing power by Benedict's and Nylander's tests. The protein was removed from the juice by colloidal iron and the clear filtrate was concentrated before applying the above tests. Since normal pancreatic juice does not

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reduce the alkaline picric acid solution, quantitative estimations of the reducing power of the juice were made by the method proposed by Lewis and Benedict for the estimation of the blood-sugar. For the same reason also it is permissible to use this method as a qualitative test for the detection of a reducing substance in the juice after the administration of phlorhizin. This was done in a few cases in which the amount of juice was too small to permit of other tests being applied.

Table I gives the results of the experiments in which the dextrose content of the pancreatic juice of normal and phlorhizinized dogs was determined. It will be seen that the percentage of reducing substances in the juice is considerably lower than that of the blood at the same time. The injection of the secretin had apparently no effect on the amount of reducing substance in the blood, and the period of starvation previous to the experiment was evidently long enough to prevent a hyperglycaemia from developing during the anaesthesia.

Attention should be called to experiment 8 in which no reducing substance appeared in the pancreatic juice following the administration of phlorhizin. The urine was loaded with sugar at the time. I can offer no explanation for this negative result. Experiment 20 is also of interest since it shows the presence of sugar in the pancreatic juice of a normal dog whose blood showed a high percentage of sugar. This animal had not been starved.

A pancreatic fistula was made in one dog after the method devised by Pavlov. The juice obtained from the fistula after the injection of 50 cc. of 0.4 per cent hydrochloric acid into the stomach or after feeding with meat was found to be free from reducing substance. The juice obtained under similar conditions, but after the administration of phlorhizin gave reduction with both Nylander's and Benedict's tests, and dextrosazone crystals were obtained with the phenylhydrazine test. The amount of dextrose present in this juice was estimated to be about 0.035 per cent.

The gastric juice

The experiments on the gastric secretions were made on dogs possessing a Pavlov stomach.[1] The juice secreted by the small stomachs after feeding with meat, was collected hourly both under normal conditions and following the administration of phlorhizin, which was given, in 2 gram doses, dissolved in 1.2 per cent sodium carbonate solution.

[1] These were generously loaned me by Professors Carlson and Luckhart of the Physiological Laboratory of Chicago University, and I wish to express my thanks for the assistance given me by these gentlemen.

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The amount of juice obtained in this way is usually small and it was therefore necessary to employ a test capable of detecting a low concentration of reducing substance in a small amount of fluid. Since normal gastric juice does not reduce the alkaline picric acid mixture as recommended by Lewis and Benedict for the estimation of blood-sugar, it was

TABLE 2

The reducing properties of the gastric juice of normal and phlorhizinized dogs

DATE	HOURLY SAMPLES	DOG B		DOG T		DOG C†	
		Qualitative dextrose test	Per cent dextrose	Qualitative dextrose test	Per cent dextrose	Qualitative dextrose test	Per cent dextrose
11/17	No. 1	0		0		0	
	Phlorhizin given						
	No. 2	+	*	+	0.03	+	*
	No. 3	+	0.025	+	0.025	+	0.035
	No. 4	+	0.035	+	*	+	*
	No. 5	+	*	0		+	*
12/3	No. 1	0		0		+	0.02
	No. 2	0		0		+	0.03
	Phlorhizin given						
	No. 3	+	0.033	0		+	0.05
	No. 4	+	0.04	+	0.038	+	0.06
	No. 5	+	0.04	+	0.03	+	0.045
12/13	No. 1	0	0	0		0	
	No. 2	0		0		0	
	No. 3	0		0		0	
	No. 4	0		0		0	
	No. 5	0		0		0	

† Dog C possessed a Heidenhain stomach.

* Denotes that enough juice was not obtained to make a quantitative sugar determination.

possible to use the reaction as a test for the appearance of reducing substance in the gastric juice following phlorhizin administration. The test is not specific for sugar and is perhaps faulty in other regards, nevertheless, it is so delicate that a very low percentage of a reducing substance can be detected in a very small quantity of fluid. The picric acid precipitates any mucus that may be present and the filtrate on boil-

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ing with a little sodium carbonate solution turns red if a reducing substance is present. I have never observed a similar reaction in normal gastric juice but have obtained it quite uniformly in the gastric juice of dogs which were under the influence of phlorhizin. The gastric juice collected from a dog with a Heidenhain pouch, on one occasion, gave slight reduction with the above reagent. Its reducing power increased, however, when phlorhizin was given. A test made on a subsequent day failed to show reduction. The gastric juice from the Heidenhain pouch of a goat (which was kindly loaned me by Professor Carlson) reduced the picric acid reagent, but did not reduce Benedict's reagent. Phlorhizin did not produce any change in the juice of this animal in the one experiment.

The results of the experiments on the gastric juice are given in Table 2.

In each of the experiments, after a sufficient amount of the juice had been collected to make quantitative estimations of the reducing power, by means of the Lewis Benedict method, the remaining samples were combined and after freeing the mixed juice of mucus, the filtrate was concentrated and tested with Benedict's reagent. Reduction occurred with the juice obtained while the animals were under the influence of phlorhizin. These results indicate that phlorhizin affects the gastric glands in the same way as it affects the pancreas.

The saliva

Some of these experiments were done on the same animals that were used in the experiments on pancreatic secretion. In such cases the saliva was obtained before the administration of the secretin. The submaxillary gland was made to secrete by stimulation of the chorda tympani and the saliva was collected by means of a cannula inserted into Wharton's Duct. The reducing power of the saliva of normal dogs and of animals poisoned with phlorhizin was tested as in the experiments on the gastric and pancreatic juice. In the saliva of normal dogs I have never found sugar save in one case (experiment 20) in which the blood-sugar was 0.18 per cent. The amount present in the saliva in this case was estimated at about 0.04 per cent and it is important to note that the pancreatic juice of this dog also reduced Benedict's reagent. This finding is in harmony with the discovery by Carlson and Ryan that the saliva of cats under ether anaesthesia may contain sugar (3).

The figures in table 3 give the results of the experiments. Out of nine experiments five are absolutely negative. Two of these are ex-

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periments in which sugar was found in the pancreatic juice. In the positive experiments the amount of reducing substance in the juice was very small. The fact that I found reducing properties in the juice of some phlorhizinized animals and did not find them in normal animals with normal blood-sugars is, I believe, significant.

In order to determine whether the action of phlorhizin on the various glands of the body is independent of its action on the kidney, I ligated the renal vessels in two dogs previous to the administration of the drug. Pancreatic juice subsequently obtained was examined for its reducing power. In one experiment in which a good flow of juice was obtained reduction occurred with Benedict's reagent. In the other experiment

TABLE 3

The influence of phlorhizin on the reducing properties of saliva. Obtained from a cannula in Wharton's duct of a dog

NUMBER OF EXPERIMENT	DATE	STARVATION PERIOD	PICRIC ACID TEST	BENEDICT'S TEST	PER CENT REDUCING SUBSTANCE IN SALIVA	PER CENT DEXTROSE IN BLOOD
2	10/28	3	0	0		0.075
3	11/1	2	0	0		0.12
12	11/16	2	+	+	0.02?	0.08
14	11/24	2	0			
17	12/8	1	+		0.035	0.08
21	12/10	0	+		0.037	0.13
22	12/11	2	+	+		0.085
23	12/14	2	+		0.03	
24	12/14	2	0			

sufficient juice only was obtained to make a qualitative test by the picric acid method, the result being positive.

The results of these experiments taken along with the observations of previous investigators of the presence of sugar in the bile and sweat of phlorhizinized animals, indicate that phlorhizin does not act on the renal cells alone. It is true that sugar is found in far greater concentration in the urine during phlorhizin poisoning than in the blood or in the above mentioned secretions, but the same is true of the glycosuria produced by the hyperglycaemia following pancreatectomy and the intra-venous injection of sugar. In one case in which I injected sufficient sugar into the femoral vein to produce a hyperglycaemia of 0.5 per cent the urine contained over 4 per cent of sugar and the pancreatic juice obtained a few minutes later, contained 0.33 per cent of reducing sub-

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stance. In this case it would hardly be thought that the sugar acts on the kidney in a different way than on the pancreas. The method by which the kidney concentrates the sugar in the urine may very well be the same in the glycosurias which follow phlorhizin poisoning and in those which depend on hyperglycaemia.

The fundamental nature of the action of phlorhizin is still unknown. The blood-sugar, as suggested by Woodyatt (4) may be considered as being in a state of equilibrium with the sugar of the cells and this again with sources (glycogen and protein) from which it may be formed.

In other words, we may think of the sugar as exerting a partial pressure in all the cells and fluids of the body just as do O_2 and CO_2. Any factor which reduces the partial pressure in one locality will eventually reduce its pressure in all parts of the body. Phlorhizin produces a change in the partial pressure of the sugar in the body, as it were, and wherever sugar can escape it does so. The chief expression of the phlorhizin action is in the kidney because in this organ there is a highly developed mechanism for the concentration of substances excreted from the blood into the urine. The methods by which phlorhizin makes the kidney cells, and the cells of other secreting glands permeable to sugar are, no doubt, alike.

SUMMARY

The normal pancreatic, gastric, and salivary juices of the dog do not contain an appreciable amount of reducing substance. The pancreatic and gastric juices and sometimes the salivary juice of dogs rendered glycosuric by phlorhizin, contain a reducing substance. In the case of the pancreatic juice, this has been shown to be dextrose.

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THE DESTRUCTION OF HORMONES, PROENZYMES AND ENZYMES BY ULTRA-VIOLET RADIATION

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During the past decade several observers have exposed enzymes to ultra-violet radiation and determined the relation between the length of time of exposure and the decrease in the activity of the enzymes. Dreyer and Hanssen (1) showed that the destruction follows the law for unimolecular reactions. Schmidt-Neilson (2) using a 1 per cent solution of rennin came to the same conclusion regarding the destruction by ultra-violet radiation. Green (3) was among the first to show the destructive effect of the short wave lengths of the spectrum on enzymes. It has been recognized in photography since the time of Bunsen and Roscoe that the relation between the exposure of silver salts to light and the amount of silver reduced by the action of light without further treatment is linear, that is, the mass of silver reduced is proportional to the energy applied. According to Faraday's law the amount of silver deposited upon the cathode, when an electric current is passed through a solution of silver salt, is proportional to the amount of current passed, that is, to the energy applied. It has been shown that this same relation exists between the amount of current passed and the destruction of enzymes (4). In view of these facts it would seem that the relation between the decrease in enzyme activity and exposure to ultra-violet radiation should be linear. The experiments reported in this paper were carried out to see if this was true.

Method. Five cubic centimeters of the solution to be exposed were introduced into a circular glass vessel 5 cm. in diameter and 1 cm. deep. This was covered during the exposure with a quartz plate 2 mm. thick and partially immersed in running water under a quartz mercury-vapor burner operating at 140 volts, 3.3 amperes, 2400 cp. Previous to the exposure the solutions were made perfectly clear by filtration. Colored solutions were rendered colorless by filtering through a thin layer of animal charcoal.

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HORMONES

Adrenalin. A colorless commercial preparation of adrenalin chloride was diluted 1 to 15 with distilled water. Five cubic centimeters of the clear solution were introduced into the glass vessel and exposed to the radiation for 20 minutes at a distance of 5 cm. This was removed and another 5 cc. was introduced and exposed for 40 minutes. Similarly another 5 cc. was exposed for 60 minutes. Three cubic centimeters of the unexposed solution were injected into the jugular vein of an etherized dog while the arterial pressure was being recorded by means of a mercury manometer. After the blood pressure had returned to normal 3 cc. of the solution exposed for 20 minutes were injected. Similarly 3 cc. of the solutions exposed for 40 and 60 minutes respectively were

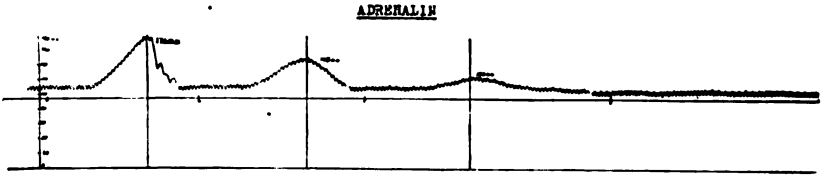


Fig. 1. The destruction of adrenalin by ultra-violet radiation. At *a*, 3 cc. of unexposed solution were injected; at *b*, 3 cc. previously exposed for twenty minutes; at *c*, 3 cc. previously exposed for forty minutes; at *d*, 3 cc. previously exposed for sixty minutes.

Length of Exposure	*Rise of Blood Pressure in mm. Mercury*	*Decrease in rise of Blood Pressure per 20' intervals of exposure of Adrenalin*
0	66	28
20	38	28
40	10	
60	0	

injected. The blood pressure records may be seen in figure 1. At *a*, 3 cc. of the unexposed adrenalin solution were injected; at *b*, 3 cc. of the solution previously exposed for 20 minutes; at *c*, 3 cc. previously exposed for 40 minutes; at *d*, 3 cc. previously exposed for 60 minutes. The injection of the unexposed solution caused a rise in blood pressure of 66 mm. of mercury; the solution exposed for 20 minutes caused a rise of 38 mm. of mercury; that exposed for 40 minutes a rise of 10 mm. of mercury, and the solution exposed for 60 minutes caused no rise in the blood pressure. The destructive effect on adrenalin of the 20 minute exposure is represented by the difference in the rise of blood pressure between 66 and 38 mm. Hg. or 28 mm.; that of the 40 minute exposure

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by the difference between 66 and 10 mm. Hg. or 56 mm. which is also a decrease of 28 mm. per 20 minute interval of exposure. In all 10 experiments similar to the one described were performed. In three of these the rate of destruction of the adrenalin was absolutely proportional to the length of exposure, while the remaining seven deviated from absolute proportionality by 5 to 15 per cent.

Secretin. This was prepared by boiling the hashed mucosa of the intestines of dogs or of cattle with 0.4 per cent hydrochloric acid, the acid extract was neutralized while boiling with 1 per cent sodium hydroxide and filtered. Five cubic centimeters of the clear filtrate were in-

TABLE I

Rate of destruction of secretin by exposure to ultra-violet radiation

Secretin

LENGTH OF EXPOSURE	NUMBER OF DROPS SECRETED IN FIVE MINUTES	DECREASE IN NUMBER OF DROPS SECRETED PER THIRTY MINUTE INTERVALS EXPOSURE OF SECRETIN
minutes		
0	15−	
30	12−	3
0	14	
60	10−	2
0	16−	
90	8+	2
0	15−	
120	6−	2+
0	14−	
150	3−	3
0	15	
180	0	3−

troduced into the glass vessel and exposed to the radiation from the burner for 30 minutes at a distance of 5 cm. This was removed and replaced by 5 cc. of fresh solution which was exposed for 60 minutes. Similarly solutions were exposed for 90, 120, 150, and 180 minutes. Four cubic centimeters of each of the solutions thus exposed were injected into the jugular vein of an etherized dog and the number of drops of pancreatic juice caused to flow in five minutes beginning with the time of the first drop were counted. Previous to the injection of each exposed solution an injection of 4 cc. of the unexposed solution was made to serve as a control. The effect of the unexposed solution was always permitted to wear off before the injection of the exposed solution took place. In Table I it may be seen that in the 5-minute interval

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after the injection of the solutions of secretin previously exposed for 30, 60, 90, 120, and 180 minutes respectively, 12−, 10−, 8+, 6−, 3−, and 0 drops of pancreatic juice were secreted. It may also be seen that the control for each of these solutions shown after 0 time exposure were fairly constant being 15−, 14, 16−, 15−, 14− and 15 drops of pancreatic juice secreted in the 5-minute intervals. The table also shows that the rate of decrease in the activity of the secretin is represented by a decrease in the flow of pancreatic juice of approximately 2 drops for each 30-minute interval of exposure of the secretin. Six experiments similar to the one described were carried out with comparatively little difference in the results.

Cholagogues. The gall bladder of a dog was tied off and a cannula was introduced into the common bile duct. Fifty cubic centimeters of bile were collected as the result of repeated injections of ox bile into the jugular vein. The bile was filtered through a thin layer of animal charcoal and rendered somewhat more colorless than the original bile. Five cubic centimeters of the filtered bile were exposed to the radiation from the quartz mercury-vapor burner in the glass vessel at a distance of 5 cm. for 10 hours. Four cubic centimeters of the unexposed bile were injected into the jugular vein of a dog having a cannula in the common bile duct. The rate of flow was increased by the injection of this amount of the unexposed bile from 5 drops to 20 drops in the 5-minute interval after the injection beginning with the time of the first drop. Four cubic centimeters of the bile exposed for ten hours were injected. This increased the rate of flow from 4 drops to 18 drops in the 5-minute interval after the injection. From the results of this typical experiment it may be concluded that exposure to ultra-violet radiation has no effect on the cholagogic properties of bile. Cholic acid prepared according to the method of Plattner was tried in the same manner as the bile with a similar result.

ENZYMES AND PROENZYMES

Trypsin and Trypsinogen. One hundred cubic centimeters of clear pancreatic juice were collected from the cannula in the pancreatic duct of a dog as the result of the repeated injections of secretin into the jugular vein. This amount was divided into two portions of 50 cc. each. To one portion 2 cc. of enterokinase were added. This 50 cc. was the solution of trypsin used in the experiments to be described and the remaining 50 cc. the trypsinogen solution used.

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Six portions of 5 cc. each of the trypsin solutions were introduced into the glass vessel and exposed to the radiation at a distance of 10 cm. for 10, 20, 30, 40, 50, and 60-minute intervals respectively. Mett's tubes made of 10 per cent gelatin colored with congo red were placed in the solutions thus exposed and permitted to digest for 24 hours at 20°C.



Fig. 2. The destruction of trypsin, trypsinogen, pepsin, and enterokinase by exposure to ultra-violet radiation.

In figure 2 under "trypsin" is shown a photograph of the tubes of a typical experiment. Tube "n" had been in the unexposed solution, tubes 1, 2, 3, 4, 5, and 6 had been in the solutions exposed to the radiation for 10, 20, 30, 40, 50, and 60-minute intervals respectively. The light portion of the tubes represents the extent of digestion, the dark portion the undigested gelatin. It may be seen that the tryptic activity is decreased by exposure to the radiation and that the rate of this decrease is proportional to the length of time of exposure.

The trypsinogen solution was exposed to the radiation as the trypsin had been. Two drops of enterokinase were added to 3 cc. of each of the exposed solutions and to 3 cc. of the unexposed solution. Mett's tubes were introduced into the solutions. After the digestion had proceeded at room temperature for 24 hours the tubes were photographed (see fig. 2 under "trypsinogen.") The rate at which the trypsinogen is destroyed by the radiation is much slower than that of the trypsin. The trypsin was completely destroyed after 60 minutes exposure while the exposure of the enzyme in the zymogen state for the same period of time decreased its activity about 50 per cent.

Pepsin. Two grams of a commercial preparation of pepsin were dissolved in 100 cc. of distilled water. This solution was filtered and portions of 5 cc. each of the clear filtrate were introduced into the vessel

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and exposed at a distance of 5 cm. for 10, 20, 30, 40, 50, and 60-minute intervals respectively. Two cubic centimeters of each of the exposed solutions and 2 cc. of the unexposed were acidified with hydrochloric acid to the extent of 0.3 per cent. Mett's tubes, made of egg white, were introduced into the solutions and the preparations were placed in a thermostat at 38°C. for 24 hours. In figure 2 under "pepsin" is a photograph of the tubes. It may be seen that the peptic activity was decreased by the exposure and that the rate of this decrease was proportional to the length of time of exposure.

Enterokinase. The mucosa of intestines of dogs as well as of cattle was gently scraped with the handle of a scalpel. This scraping was extracted with 0.9 per cent sodium chloride, centrifugalized and filtered until clear. Portions of 5 cc. each of the clear filtrate were introduced into the glass vessel and exposed to the radiation at a distance of 5 cm. for 2, 4, and 6-minute periods. Four drops of the unexposed enterokinase were introduced into a tube containing 2 cc. of trypsinogen. Similarly 4 drops of the solutions exposed for 2, 4, and 6 minute periods were introduced into tubes containing 2 cc. each of trypsinogen. Mett's tubes made of gelatin were introduced into the solutions and allowed to digest at room temperature for 24 hours. In figure 2 under "enterokinase" is a photograph of the tubes. The power of the enterokinase to activate trypsinogen was entirely destroyed after 6 minutes' exposure to the radiation.

Ptyalin. Fifty cubic centimeters of fresh saliva were centrifugalized and filtered until clear. Portions of 5 cc. each were exposed to the radiation at a distance of 5 cm. for 10, 20, 30, 40, 50 and 60 minute intervals. Two cubic centimeters of the unexposed solution were added to a 2 per cent starch paste at 38°C. The preparation was allowed to remain in the water bath at this temperature for 10 minutes. The solution was then boiled and the amount of sugar determined by Pavy's method. The amount of sugar produced by the action of 2 cc. of each of the exposed solutions was determined in a similar manner. The results of a typical experiment may be seen under "ptyalin" in Table II.

Amylopsin. Experiments similar to those made upon saliva were carried out with a clear solution of pancreatic juice collected from a dog. In Table II under "amylopsin" are given the results of a typical experiment.

It may be seen that the activity of ptyalin and of amylopsin is decreased by exposure to ultra-violet radiation and that the rate of this decrease is about 20 per cent per 5-minute interval of exposure for the ptyalin and per 10-minute interval of exposure for the amylopsin.

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TABLE II

The destruction of ptyalin and amylopsin by ultra-violet radiation

LENGTH OF EXPOSURE	SUGAR PRODUCED BY ACTION OF PTYALIN	DECREASE IN ACTIVITY OF PTYALIN PER FIVE MINUTE INTERVAL OF EXPOSURE	SUGAR PRODUCED BY ACTION OF AMYLOPSIN	DECREASE IN ACTIVITY OF AMYLOPSIN PER FIVE MINUTE INTERVAL OF EXPOSURE
	mgms.	*per cent*	*mgms.*	*per cent*
0	17.4		14.0	
5	13.8	20		
10	11.0	19−	10.9	22
15	9.0	17−		
20	4.4	18+	7.9	22
25	2.5	17+		
30	1.7	15+	5.5	20
35	+	20−		
40	0.0		1.8	18
45				
50			+	20−

In all the experiments reported in this paper the substances were exposed in a glass vessel covered with a quartz plate. The substances were also exposed in the same vessel and under the same conditions except the vessel was covered with a clear glass plate 5 mm. thick instead of the quartz plate. When this was done none of the substances were affected after many hours exposure. It had been determined that the glass cover used did not transmit wave lengths shorter than 313 $\mu\mu$ hence these shorter wave lengths destroyed the substances exposed. It had also been determined that only wave lengths 302 $\mu\mu$ and 297 $\mu\mu$ in the spectrum of the quartz mercury-vapor burner used were effective in coagulating protein. It is reasonable to assume that these wave lengths caused the destruction of the substances exposed.

CONCLUSIONS

Hormones, proenzymes, and enzymes are destroyed by exposure to ultra-violet radiation. The rate of this destruction is proportional to the amount of energy applied. The specific wave lengths causing the destruction are 302 $\mu\mu$ and 297 $\mu\mu$. The cholagogic activity of bile is not affected by exposure to ultra-violet radiation.

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A NOTE ON THE EXCITATION OF THE PHRENIC NERVE BY THE ACTION CURRENT OF THE HEART

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The stimulation of the phrenic nerve by the action current of the heart has been seen by a number of investigators. Stewart and I observed some years ago that it was not necessary to open the chest cavity or to disturb the heart in any way in order to elicit contractions of the diaphragm at each beat of the heart (1). The interpretation placed upon our experiments at that time was that the change in excitability of the phrenic nerve due to changes in its blood supply, was the important element in the onset of its excitation by the action current of the heart. Our reasons for this view were:

1. That without any change in blood pressure or the relative positions of the heart and the phrenic nerves, the phenomena of excitation would appear.

2. In all experiments in which the phrenic nerve became excitable to the action current of the heart the blood supply of the upper portion of the phrenic nerve was cut off by ligation of the internal mammary artery at its source. The excitation of the nerve was manifested only some minutes after the artery was ligated. If the artery was occluded for a still longer time, the nerve again became inexcitable to the action current of the heart.

3. If the nerve had ceased to respond during the time the artery was occluded, it would again become excitable some time after the ligature was removed from the artery and the circulation to the nerve restored. The excitation would persist for a time and again cease as the nerve presumably more nearly approached its normal state, due to the access of oxygenated blood to it.

To these considerations we might add that in all of the cardiac conditions in which, as revealed by the electrocardiogram, there is any interference with the normal conducting mechanism of the heart or any disturbance of its electrical relations, excitation of the phrenic

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nerve by the action current of the heart so far as my knowledge goes, has not been observed. In pericarditis with effusion, the short circuit for the action current of the heart is probably somewhat affected, the increased fluid perhaps providing a better return than usual. In some of the clinical conditions of the heart which have been investigated a worse short circuit for its action current, as Langendorff (2) suggested might be expected occasionally to occur. If this occurrence of a worse short circuit is the reason for the appearance of the excitation of the phrenic nerve by the action current of the heart, we would expect the twitching of the diaphragm to occur at times in clinical cases. It does not appear, therefore, that the change in the heart itself is the real condition, or, at least, the most important condition for the excitation of the phrenic by the action current of the heart.

The threshold value of stimulation of the phrenic nerve under ordinary normal conditions must be considerably above the ordinary effectiveness of the heart's action current, for twitching of the diaphragm is not observed in any cases of hypertrophy or in any other condition of the heart in which the magnitude of the action current is greater than normal.

I am, therefore, still of the opinion that the important element in the excitation of the phrenic nerve by the action current of the heart is the change in the excitability of the nerve itself. It seems probable that the accumulation of carbon dioxide may play the same rôle in increasing the excitability of the phrenic nerve that it does in the treppe of skeletal muscles (3). The inexcitability of the phrenic under all ordinary, and many extraordinary, conditions to the action current of the heart is a phenomenon so familiar as to escape comment. But one has only to reflect upon what life would be like if there were constantly present in the speech or song of all of us, abrupt, jerky tremors similar to those appearing in hiccough, to imagine some reason why the nerve is ordinarily inexcitable to the action current of the heart.

The new observation which is recorded here brings out, in what is to me a most instructive way, the difference in the speed of conduction of impulses in nerve tissue and in the heart itself. The diaphragm could be seen to contract before the ventricular systole became apparent to the eye. Perhaps other workers may be led to look for and record similar conditions.

January 19, 1916. A cat was used for a blood pressure experiment, but no drugs, aside from the ether for anaesthesia, were used. At the

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close of the experiment, the trachea was clamped until the respiration stopped and the blood pressure fell very low. The respiration and heart beat did not start up again when artificial respiration alone was used, and the thorax was then opened. The heart was started by direct massage, after removal of the pericardial sac and clamping the thoracic aorta just above the diaphragm. The heart soon started under artificial respiration and direct massage, and the beat became strong and regular. The artificial respiration was then intermitted and the heart allowed to beat as long as it would. As the ventricular beats became slower, the auricular beats continuing at a greater rate than the ventricular, the left side of the diaphragm began to twitch at each beat of the ventricle. The heart was lying against the left phrenic nerve about 2 cm. above the diaphragm. The auricular beats were not sufficient to cause the twitching of the diaphragm, but only the ventricular beats. The diaphragm did not twitch on both sides, but only on the left. Nor did it twitch when the apex of the heart was moved over to the right side of the chest cavity, where it did not come in contact with the left phrenic nerve. The spontaneous beat of the ventricle was sufficient to excite the phrenic nerve, but the beat caused by touching the right ventricle with the point of a knife would not bring about any contraction of the left side of the diaphragm. The diaphragm could be seen to twitch strongly just before the systole of the ventricles became apparent to the eye. The interval was a fraction of a second, but long enough to admit of no doubt. The ventricular beats began to come in groups (Luciani's groups) toward the end, and the diaphragm showed a rhythm synchronous with that of the ventricles.

The tentative interpretation, for part of which I am indebted to Dr. H. B. Williams, is that the action current of the ventricles stimulated the left phrenic nerve. Owing to the fact that the action current precedes the sound of the heart due to the contraction of the ventricles one may suppose that the nerve was stimulated somewhat before the ventricular systole became apparent. The diaphragm reacts more quickly than the ventricular muscle; and the 2 cm. or so of phrenic nerve conducted the stimulus to the muscle more rapidly than the conducting substance in the slowly beating heart conducted the impulse to the ventricular muscle. The striking thing about the matter was the fact that the left side of the diaphragm twitched before the systole of the ventricles became apparent to the eye. The clamping of the

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thoracic aorta, resulting in shutting off most of the blood supply of the lower part of the phrenic nerve, may have had something to do with its increased irritability.

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THE CHARACTER OF THE INNERVATION OF THE KIDNEY

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While the existence of vasomotor fibers to the kidneys needs no further confirmation, it seems desirable to ascertain whether the innervation of these organs is crossed or unilateral in character. By a series of quantitative measurements of the bloodflow through the left and right renal veins it has been established (1) that it is possible to markedly diminish the vascularity of the kidneys by the stimulation of the corresponding greater splanchnic nerve, as well as by the excitation of the plexus renalis and its single constituents. By the same method it has been found that the vagi nerves do not possess a vasomotor action upon the kidneys and that the renal plexus, in conjunction with the suprarenal plexus and the vagal terminals, forms the afferent path by means of which impulses gain the central nervous system.

Quite different results were obtained if the greater splanchnic nerve of the opposite side was subjected to the stimulation. At no time were we then able to incite such quick and powerful constrictor reactions as we had been able to get with the help of the preganglionic path of the same side. Thus, the stimulation of the right nerve usually led to a reduction in the vascularity of the left organ which set in only after a considerable latent period, was very variable in its amplitude and frequently disappeared long before the end of the excitation. Practically the same kind of differences were noted when the bloodflow through the right kidney was measured during the stimulation of the left nerve. It was also observed that these peculiar reductions in the renal blood-supply were generally ushered in by rather brief augmentations in the flow. As the latter developed in strict accordance with the rise in the systemic blood pressure, they were attributed to the general vascular conditions and not to a local dilator action. All these changes, but especially the initial augmentor phenomenon, led me to believe that the direct vasomotor influence of the splanchnic nerve is restricted to the

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organ of the corresponding side, and that therefore the innervation of the kidneys is unilateral in its character.

But while I clearly recognized at that time the different changes which occur under the experimental conditions just described, and while the conclusions drawn from them need no correction, I was much puzzled to find an explanation for the peculiar behavior of the kidney situated on the side opposite to the stimulation. In the last few years, however, certain data have been gathered regarding the function of the adrenal glands which, had they been obtained at a somewhat earlier date, would have greatly aided me in explaining my results. For this reason the plethysmographic experiments upon the kidneys dealt with in the succeeding pages, are intended to substantiate my earlier data and secondly, to bring them into relation with the more recent work upon the secretory activity of the suprarenal bodies.

Johansson (2) called attention to the fact that the elevation in the general blood pressure occasioned by stimulation of the splanchnic nerve, is not a simple rise and fall, but presents two summits. Bayliss (3), moreover, observed that the blood vessels of peripheral parts do not behave passively towards the changes in general blood pressure occasioned by the stimulation of the aforesaid nerve, but frequently display an independent constrictor action. Lehndorff (4) then offered the explanation that the first increase is caused by the constriction of the blood vessels innervated by the splanchnic nerve and the subsequent transfer of this mass of blood into the systemic circulatory channels. The second elevation he believed to be due partly to a greater force and frequency of the heart, and partly to a constriction of certain peripheral blood vessels. By extirpating the suprarenal bodies Elliott (5) succeeded in demonstrating that the second rise is dependent upon the outpouring of adrenalin into the circulation, the splanchnicus, as has been demonstrated by Asher (6), being the secretory nerve of this gland. The more recent experiments of von Anrep (7) have proved this conclusion to be correct, and have shown moreover that the stimulation of this nerve is also capable of inciting a constrictor reaction in the denervated kidney and hind-limb in an indirect manner by forcing a certain quantity of adrenalin into the circulation which finally enters the blood vessels of the passive organs.

The present experiments were performed upon dogs in ether narcosis. Both kidneys were enclosed in oncometers which were connected with recording pistons. The general blood pressure was registered by a mercury manometer which communicated with the left femoral artery.

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Both thoracic sympathetic nerves were placed in shielded electrodes at a point directly above their division into the splanchnicus major and sympatheticus abdominalis. The intact nerves, as well as the distal ends of the divided nerves were employed. Naturally, this procedure necessitated the piercing of the diaphragm in the region of the crura and artificial respiration. The stimulations were indicated by a signal, while the time was registered in seconds by a Jaquet chronograph. All writing levers were adjusted in the same ordinate at the beginning of the experiment. In order to avoid possible conflicts, the zero-line of the blood pressure has not been recorded upon the paper.

The precedure followed in one series of experiments consisted in stimulating the right or left nerve at intervals with a current of moderate strength and duration, the endeavor being to obtain at least some reactions, which permitted of a comparison with others of the same amplitude. In another series of tests both nerves were stimulated simultaneously with currents of practically the same strength and duration, the individual reactions being incited at relatively long intervals so as not to exhaust the secretory power of the adrenals.

The accompanying figure 1 is intended to illustrate the changes in the volume of the kidneys following the stimulation of the left splanchnic nerve. The general blood pressure represented by the uppermost record, presents an elevation which is composed of two rises. The initial increase (to c) begins after a very brief latent period and gives way about 15 seconds later to a rise of still greater amplitude (after c). As has been stated previously, the first elevation is attributable to the vaso-constriction occurring in the different organs innervated by the aforesaid nerve, and the second, to a much more general constriction of the blood vessels caused by the discharge of adrenalin into the circulation. While the first effect appeared within a second or two after the onset of the stimulation, the subsequent elevation developed only after a well marked latent period, the average duration of which amounted to 12.8 seconds. Values between 10.4 and 16.2 seconds have been obtained, the time being practically the same for the two glands.

It is possible, however, to account for these variations without much trouble, because the circulation-time in different animals shows certain normal differences, and because the dynamical conditions constantly tend to become less favorable if the experiment is continued for a long period of time. For the same reason, the rapidity with which the adrenalin is discharged diminishes somewhat in the course of an experiment of this kind, and especially if the intervals between the

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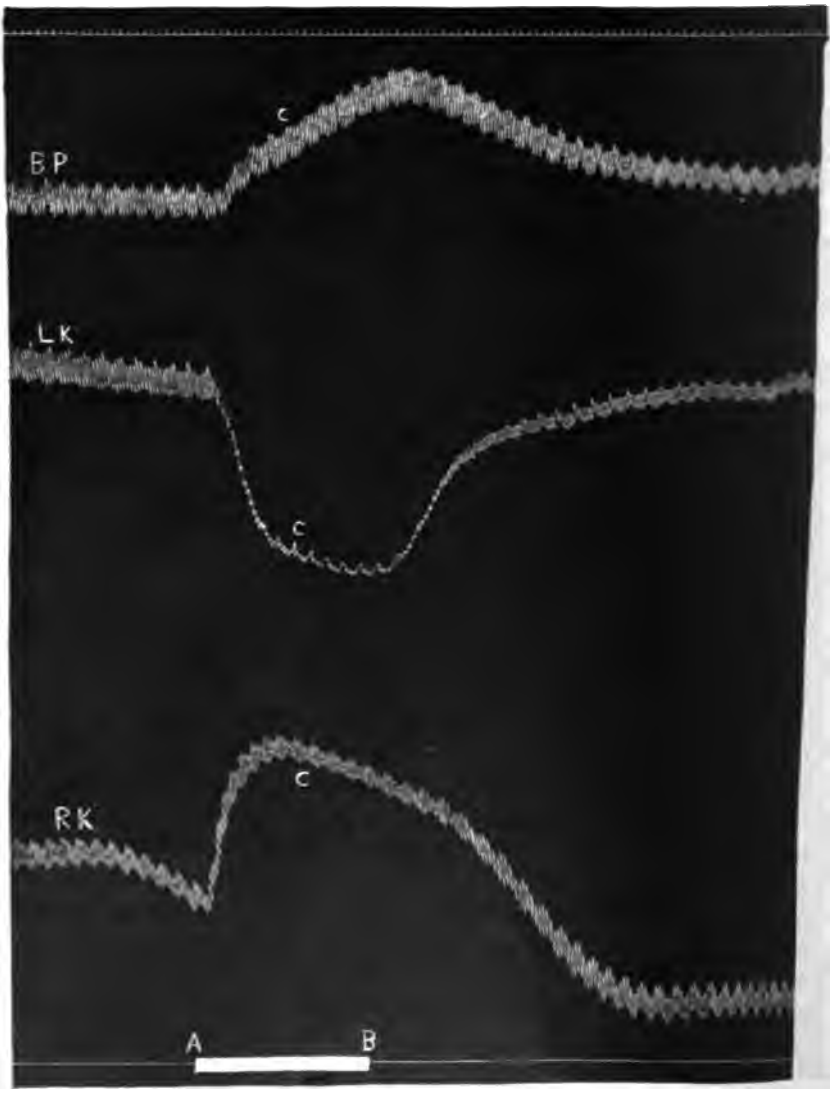


Fig. 1. Stimulation of the left splanchnic nerve (15 cm., 23 seconds)

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stimulations are not sufficiently long to permit of a complete readjustment of the secretory power of these glands. Thus, it may happen that the second summit of the rise in the general blood pressure disappears entirely, while the first elevation, although of lesser amplitude, remains clearly in evidence.

By injecting minimal doses of adrenalin into the current of the portal vein in the vicinity of the hilus of the liver (8) I have found the circulation-time between this blood vessel and the systemic arterioles to be 14.1 seconds. In a similar way I have recently determined the time consumed in the passage of adrenalin from the renal and femoral veins to the distal arterial channels. In the former case, the average value obtained was 13.8 seconds and, in the latter, 16.4 seconds, these figures agreeing very well with the preceding value of 12.8 seconds for the circulation-time between the suprarenal veins and the arterial system.

The difference in time between the femoral vein, on the one hand, and the renal and suprarenal veins, on the other, amounts to about 2.6 seconds. In a dog weighing about 12.0 kg., the distance between the more peripheral blood vessel and the orifice of the renal vein measures approximately 17 cm. If now these two values are brought into relation with one another, it becomes evident that the blood progresses through the lower portion of the inferior vena cava and its iliac tributaries at the rate of about 65 mm. in a second.

In the curve represented by figure 1, the plethysmographic tracings of the kidneys are arranged below that of the blood pressure (B P), the one of the left organ (L K) being placed above that of the right (R K). The period of stimulation is indicated by the letters *A* and *B*. It is evident that the two records pursue at first a course in opposite directions to one another, the left kidney showing a decrease in its volume and the right organ an increase. Moreover, as these changes appear almost synchronously with the stimulation, they must be regarded as the direct results of the activity of the left splanchnic nerve. We know that this nerve exerts a vasoconstrictor influence upon the left kidney, and hence, it may justly be concluded that the fall in the oncometer record is attributable to a decrease in the vascularity of this organ.

The initial rise in the tracing of the right kidney shows first of all that the left splanchnic nerve possesses no direct vasomotor influence upon this organ, because under ordinary conditions of experimentation this nerve exhibits a constrictor tendency, and because it can readily be proved that this elevation has not been caused by vasodilator changes.

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Thus, the deduction may justly be made that the right kidney remains passive at first, its increased volume being occasioned by the greater arterial influx resulting in the course of the rise in the systemic blood pressure. That this explanation is correct may readily be gathered from a study of the general character of the curve. While the rise develops very shortly after the onset of the stimulation, a longer latent period cannot rightly be expected in this case, because the general blood pressure has been registered in these experiments in the femoral artery, i.e., very close to the seat of the primary vasoconstriction. The remaining extent of the curve, however, exhibits a perfect parallelism with the height of the blood pressure.

About fifteen seconds after the beginning of the stimulation, the oncometer tracing of the right kidney exhibits a sudden decline which, in some cases, merely attains the level of the record previous to the stimulation and, in others, reaches much below this line. The left kidney retains its small volume during this period, or if a relaxation of its blood vessels has already set in, a secondary decline may result. As this belated decrease in the volume of the kidneys occurs synchronously with the second elevation in the curve of the systemic blood pressure, it must be ascribed to the action of the adrenalin which has been liberated by the left adrenal body in consequence of the excitation of its secretory nerve. Obviously, the severity of this indirect vasoconstriction of the renal blood vessels must be in complete agreement with the secretory activity of the gland. Late in the course of an experiment of this kind, and especially if the stimulations have been repeated too frequently, the decrease in the volume of the right organ becomes less abrupt and may eventually disappear altogether. It need scarcely be mentioned that the second summit may also be destroyed at any time by temporarily obstructing the suprarenal vein. The volume curve of the right kidney pursues under this condition a course parallel to the record of the systemic blood pressure.

In figure 2 the changes are depicted which result in consequence of the excitation of the right splanchnic nerve. We observe first of all the almost instantaneous decrease in the volume of the right kidney occasioned by the direct vasoconstrictor action of this nerve, and secondly, the initial increase in the volume of the left kidney which, however, is soon followed by a diminution. In this case, naturally, the right suprarenal gland is responsible for the outpour of adrenalin. It is again to be noted that the right splanchnic nerve does not exercise a direct control over the state of the vasomotor mechanism of the left kidney.

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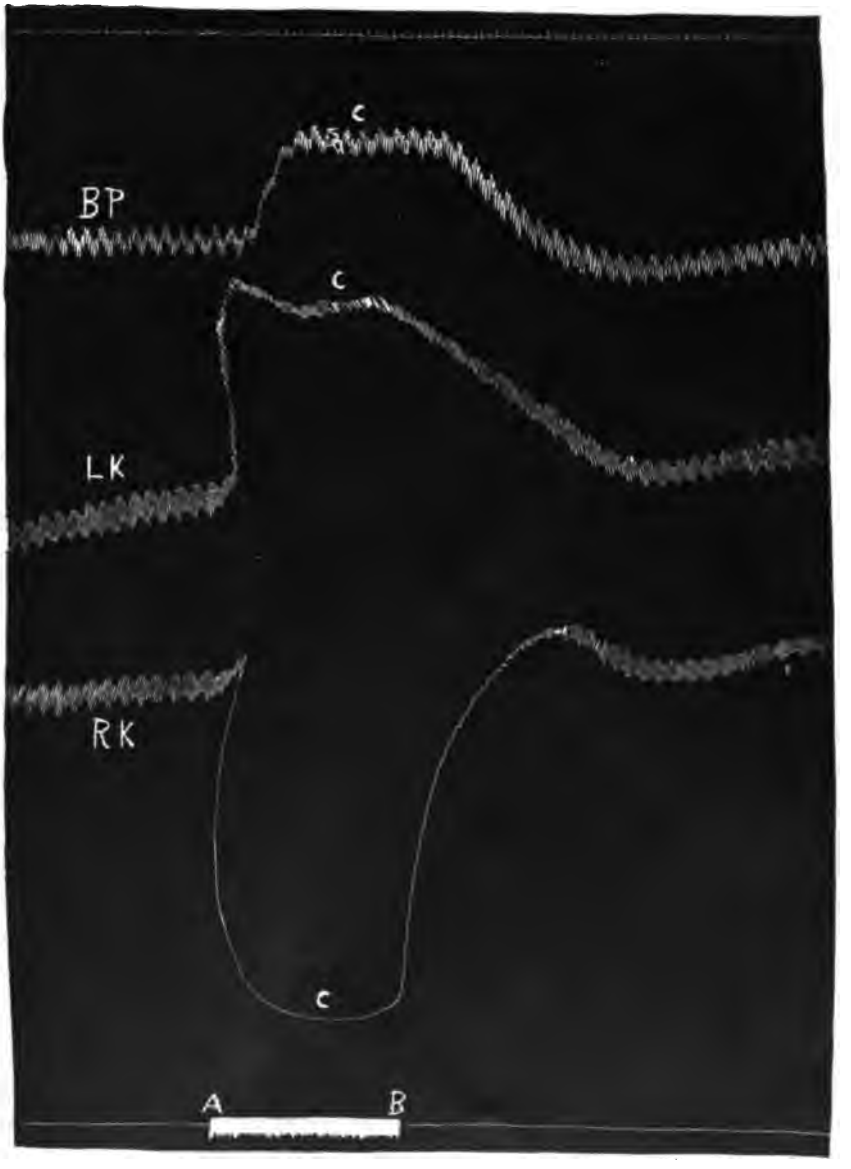


Fig. 2. Stimulation of the right splanchnic nerve (15 cm., 24 seconds)

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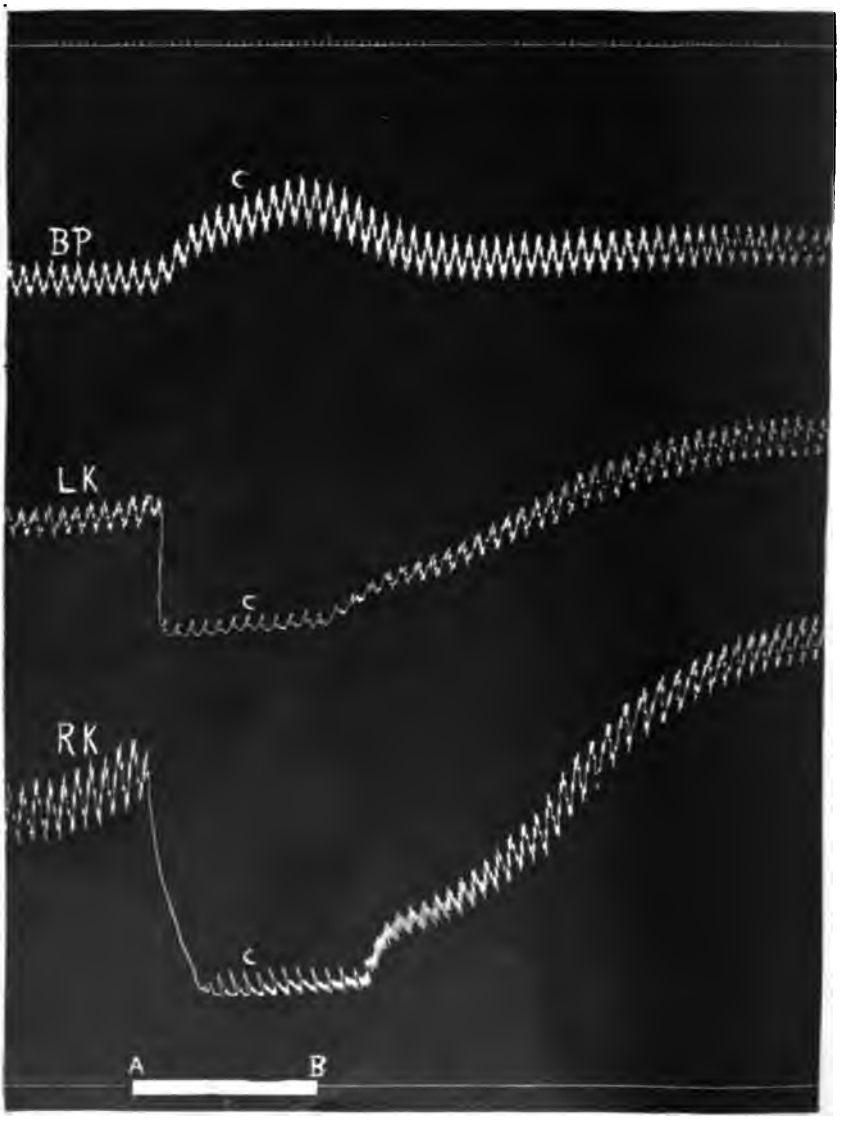


Fig. 3. Stimulation of both splanchnic nerves (13 cm., 25 seconds)

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Figure 3 is intended to serve as an example of those reactions which were obtained by the simultaneous stimulation of the left and right splanchnic nerves. The oncometric tracings now show almost immediate decreases in accordance with the bilateral constriction of the renal blood vessels. In these cases the subsequent discharge by the two adrenal bodies generally failed to augment the primary effect in a very noticeable manner, but it is possible to render the secondary reaction more conspicuous by lessening the amplitude of the primary. This end can readily be attained by decreasing the duration and strength of the stimulation; in fact, it is possible to adjust the latter in such a manner that perfectly parallel curves may be obtained from the two kidneys.

These experiments, therefore, uphold my previous contention that the innervation of the kidneys is unilateral and enable me moreover to explain the peculiar changes in the blood supply of these organs which I obtained on contralateral stimulation of the splanchnic nerves. The initial increase in the flow I have attributed correctly to the rise in the general blood pressure, because as the kidney on the opposite side is not directly affected by the stimulation, its vascularity must be subject to the changes in the arterial driving force. The subsequent decrease in the blood supply for which at that time I could offer no explanation, now finds its true solution in the secondary constriction of the renal blood vessels by the adrenalin.

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SOME OF THE GENERAL PHYSIOLOGICAL PROPERTIES OF DIAPHRAGM MUSCLE AS COMPARED WITH CERTAIN OTHER MAMMALIAN MUSCLES

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1. INTRODUCTION

The diaphragm, because of its unique position in the mammalian body, its unique structure, and its unique action, has always been an object of interest. Haller (1), writing in 1733 his "De musculis diaphragmatis dissertatio anatomica," mentions some fifty writers, beginning with Plato and Aristotle, who had set forth their views, as the result of speculation, or observation, or experimentation, concerning the action and use of this peculiar muscle. To Fabricius (2) has been ascribed the first recognition—in 1603—of the true action of the diaphragm in respiration, but Haller, with his clear-sighted vision, contributed materially to an understanding of its function, and since Haller's time the literature concerning it has been considerable. But with

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all that has been said upon its gross anatomy and its specific physiology one searches almost in vain for enlightenment regarding its histological structure, its chemistry, and the general physiological properties of its muscular tissue. It seems strange, indeed, that more thought has not been given to its general physiology, for this topic, even on slight consideration, appears promising. In importance to the individual the diaphragm ranks highest of all skeletal muscles: although its bilateral paralysis, whether from trauma or disease of the nervous system, is not rare, the continuance of life in such a condition is difficult, if not impossible. Moreover, while most skeletal muscles in the living body contract with varying degrees of intensity and at irregular intervals between which occur long periods of rest, the diaphragm from the time of birth to that of death performs day and night a continual succession of brief contractions of a fairly regular rhythm and fairly uniform in extent, alternating with brief intervals of rest. Thus this muscle, together with other respiratory muscles, holds a unique position among skeletal muscles and suggests a crude analogy with the heart. Like the heart too, the diaphragm performs during the lifetime of the individual an incredibly huge amount of work, probably more than any other skeletal muscle.

In view of these facts it might be expected that a careful study of this important muscle would reveal physical and chemical peculiarities which would distinguish it from other muscles. Such a study was planned by the senior author several years ago, and since then work upon it has been carried on from time to time, as opportunity has permitted. The results of the physical portion of the research, as here presented, justify the predictions, for it is shown that the diaphragm does possess distinctive general physiological properties.

Preliminary experiments were made with the diaphragms of the cat, the rabbit and the guinea pig, but that of the cat proved most satisfactory and was chosen for detailed study. Its behavior under the various experimental conditions has been contrasted with that of certain muscles of the leg, such as the extensor longus digitorum, the sartorius, and the soleus, and at times the muscular tissue of the heart. The extensor and the sartorius were chosen as typical pale muscles, the soleus as the type of red muscles. The diaphragm appears intermediate between these two in color. We shall see that in physiological properties color is a feature of limited significance.

We have used for our experiments only a certain part of the diaphragm, namely, a strip extending along both sides of its anterior

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median line from the bone and cartilage of the ensiform process to the central tendon. This is the strip which in the rabbit is unusually well isolated and has been widely used in recording the contractions of the muscle in respiration. In the cat it is not so much separated from the rest of the muscle, but by observing the direction of its fibers one can readily cut it out with a minimum of injury. Its average dimensions are 50 mm. in length, and from 10 to 20 mm. in breadth. It appears to consist of long parallel fibers. That the fibers are, in fact, long and extend practically from one end of the strip to the other, is indicated by the results of stimulating the muscle electrically in a manner suggested by Kühne's unipolar method. The secondary coil of an inductorium is provided with two electrodes: one formed by a metal plate, on which lies a curarized strip of the diaphragm, and the other consisting of a fine needle which is carefully brought to touch the muscle. With the interrupter of the inductorium in vibration and with an electrical current that is not too strong, the plate serves as an indifferent, the needle as an active, electrode. Under such circumstances it can be seen that the muscle contracts over an area twenty to thirty times as long as it is broad. Furthermore, after maceration for twenty-four hours in nitric acid, the strip can be torn into delicate threads running its entire length, and under the microscope these can be seen to consist of a dozen or more long muscle fibers. The sartorius of the cat is a large muscle, consisting of a thick lateral and a thin mesal paler portion. We have used only the latter. Its fibers are very long, but whether they extend actually from end to end of the muscle, we cannot say. In the extensor longus digitorum and the soleus, of which muscles the whole substance has been used for study, the fibers extend, not longitudinally, but diagonally, from an aponeurotic extension of one tendon to a similar extension of the other in the case of the extensor, and to the long tibial insertion in that of the soleus.

No one appears yet to have made an exhaustive study of the histological structure of the diaphragm and its histological relations to other muscles. Schiefferdecker (3), using human and canine diaphragms, has most nearly approached this, but his results, while intrinsically of importance, do not appear to be specifically related to our physiological facts. In both man and dog he finds scattered large muscle fibers of a more or less circular cross-section, surrounded by small fibers which appear in section polygonal and often with sharp corners. He argues that in the larger fibers the protoplasm is under a higher tension, although he does not know their specific function. In both the

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large and small fibers of the human diaphragm, while most of the nuclei lie just under the surface, a relatively larger number than is usual in other muscles lie more deeply; in the dog's diaphragm almost all the nuclei are superficial. We have examined sections of the several varieties of cat's muscles that have been used in our experiments. No very striking differences have been observed. The sections of the diaphragm have shown considerable differences in the size of cross-sections of individual fibers, but no grouping of large round, surrounded by small polygonal, fibers. The cat's fibers resemble the dog's, however, in the almost exclusively superficial location of the nuclei.

2. THE RELATION OF OXYGEN

Ehrlich (4), in his classic work on the oxygen requirements of the organism, found that after the injection of a solution of indophenol white certain groups of muscles including the diaphragm, besides the heart, the brain, and the kidney, appeared blue owing to the oxidation of the drug, and he pointed out that it is these organs, which are of the most importance to the organism, "die vor allen anderen am wenigsten reductionskräftig, d.h. relativ am besten mit Sauerstoff gesättigt sind." Bonhöffer (5), using a method that had been employed by Bernstein (6) and even before him by Yeo (7) under Kronecker's direction, found that the quickly acting pale muscles of the rabbit, guinea pig and rat reduced oxyhaemoglobin more rapidly than the slowly acting red muscles. Knoblauch (8) too, employing one of Ehrlich's methods, observed in rabbits after death in tetanus resulting from the administration of alizarin blue S, which reduces to alizarin white, that the red muscles, such as the soleus, the semitendinosus and the masseter, still remained red, while the pale muscles were blue—the explanation being found in the fact that the more rapidly fatiguing pale muscles quickly lost their reducing power, which the resistant red muscles still retained. Rehns (9) found that after injecting into mice a soluble salt of paraphenylenediamine the diaphragm oxidized the salt and hence was stained blackish during life, while all other muscles, save those of the eye and the larynx though in lesser degree, required the aid of the atmospheric oxygen to effect the oxidation. Nothing analogous appeared in the rabbit, guinea pig, and rat; nevertheless, Rehns concluded that the diaphragm is peculiar among muscles in possessing a superabundant supply of available oygen. The results of these four men suggest that muscles in relation to oxygen may exhibit two differences: They

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may differ not only in their power to reduce oxygen-containing chemical substances, but in the amount of oxygen which they contain.

We have studied the general problem in the four cat's muscles by several methods. In an endeavor to discover any differences in reducing power that might distinguish the muscles we injected strong solutions of alizarin blue S subcutaneously into ten cats and after killing the animals observed the degree of coloration of the various muscles. While the method proved not altogether satisfactory and decisive, owing to uncertainty in the distribution of the substance throughout the body, it afforded some evidence that the diaphragm possesses the greatest power of reduction of all the muscles. We next turned to a simpler method *in vitro*, using oxyhaemoglobin as the indicator, and here we obtained very striking and uniform differences in reducing power. A solution was prepared by mixing 1 part of cat's blood with 99 parts of distilled water. To this was added enough sodium chloride to bring the mixture to an 0.8 per cent solution of the salt, and 7 cc. of this were placed in each of four flat bottles. The animal was killed by decapitation and the four muscles were quickly dissected out. One gram of each was rubbed up in 0.7 grams of powdered glass and placed at once in the blood solution, which was then covered by a layer of olive oil to exclude the air. (When these experiments were performed we were not acquainted with Vernon's work on the solubility of air in fats—Proc. Roy. Soc., B, 1907, lxxix, 366. We have no reasons, however, to believe that the facts which he presents would alter our general results.) The finely pulverized muscle and glass settled to the bottom, and the oxyhaemoglobin of the supernatant solution became progressively reduced from below upward. The specimens were observed with the direct vision spectroscope at frequent intervals and the time was noted at which in each the spectrum of oxyhaemoglobin gave place to that of reduced haemoglobin. In order to insure exactness of measurement observation was confined to the layer of the solution that was in immediate contact with the muscles and the width of which just equalled the length of the slit in the spectroscope. The results of the thirteen experiments performed are given in table 1.

The reducing power of the four muscles is seen to be greatest in the diaphragm and to descend in the order: diaphragm, extensor, sartorius, soleus; the average time required for the last being more than four times that of the first in the series.

The same general result was confirmed by means of extracts of the muscles and methylene blue in experiments which were conducted as

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follows: The four muscles were removed as quickly as possible after the death of the animal, and a gram and a half of each was weighed out. Each of these portions was ground in a mortar with 2 grams of glass powder for 10 minutes. The four preparations were placed in 30 cc. of distilled water, and the mixtures were thoroughly stirred and allowed to extract for one hour at room temperature. At the end of this time they were centrifuged for at least 15 minutes, and subsequently, if a deposit had appeared on the surface, the supernatant fluid was filtered. Five cubic centimeters of each of these extracts were added

TABLE 1

Reducing power of muscles, as measured by the number of minutes required to reduce oxyhaemoglobin

NUMBER OF EXPERIMENT	DIAPHRAGM	EXTENSOR	SARTORIUS	SOLEUS
1	19	56	55	372
2	18	16	50	280
3	25	28	31	55
4	24	22	53	144
5	44	21	88	99
6	44	40	45	80
7	52	65	70	246
8	23	37	51	47
9	25	40	42	44
10	48	22	54	117
11	38	41	42	77
12	24	30	58	109
13	27	27	38	138
Average.....	32	34	52	139
Percentage.....	100	106	162	434

to an equal quantity of a weak aqueous solution of methylene blue in a test tube, the mixture was covered with paraffin oil, and the rate of reduction was judged by the disappearance of the blue color, which began at the bottom and progressed upward. Decoloration of most of the solutions was complete in from 1 to 3 hours with most of the muscles, but with the extract of the soleus it required some 2 days. In addition to the test tubes containing the extracts of the four muscles there were always two controls. The first contained a sample of the solution of methylene blue but no muscle extract; it underwent no change of color. The second control contained the dye and the usual quantity of one of the muscle extracts, but the mixture was boiled for a half minute; its

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color became slightly lighter immediately upon adding the extract and before boiling, but thereafter there was no change. Seven observations were made with muscles taken from 6 cats. The order in which the several extracts reduced the dye is given in table 2.

It is here seen that, as with oxyhaemoglobin, the diaphragm possesses the greatest, the soleus the least, reducing power; the two pale muscles hold an intermediate position with the sartorius slightly in the lead.

The mechanism of the process of biological reduction is not yet agreed upon. Harris and Creighton (10) have presented data which they interpret as evidence in favor of the existence of a specific reductase in the organs of the animal body. If we accept their interpretation we may say that the diaphragm possesses the most reductase, the soleus

TABLE 2

Reducing power of muscles, as measured by the order in which extracts of the muscles reduce methylene blue

NUMBER OF EXPERIMENT	ORDER OF REDUCTION	
1	Di Sar Ext Sol	
2	Di Sar Ext Sol	
3	Di Ext Sar Sol	Ext. and sar. reduced almost simultaneously
4	Di Sar Ext Sol	
5	Di Sar Sol Ext	
6	Di Ext Sar Sol	Same extract as in Exp. 5. Ext. and sar. reduced simultaneously
7	Di Ext Sar Sol	

the least. But, whatever the mechanism, the facts show that there exist pronounced and constant differences in reducing power in different muscles and that, of all the muscles studied, the cells of the diaphragm possess the greatest power to utilize the oxygen of their environment. In this respect the diaphragm is a superior physiological mechanism.

In an endeavor to learn whether Rehns' conclusion as to the existence of a reserve supply of oxygen in the diaphragm of the mouse holds good for another mammal, we have made fifteen autopsies on cats, after injecting solutions of the hydrochloride of paraphenylenediamine during life into various places—subcutaneously, the carotid artery, the external jugular and femoral veins, the thoracic cavity, the abdominal cavity, and immediately over the site of the three leg muscles that have been studied. The presence of available oxygen at any place

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would be indicated by the drug there becoming dark purple or blackish. No injections into the blood vessels resulted in staining of the muscles or other tissues. Injections into the thorax or abdomen stained the muscles at the point of injection and seemed to indicate a selective action toward the drug on the part of the diaphragm, but the results were not clear cut, the decision was left doubtful, and the method was abandoned. A few observations on the results of mixing a solution of paraphenylenediamine with extracts of muscles, prepared in the same manner as in the above experiments with methylene blue, were also indecisive, and we are, therefore, forced to leave unsolved this part of our problem. We may, however, point out that even Rehns was unable to confirm in the rabbit, guinea pig and rat his findings on the mouse, and that thus his generalization as to the diaphragm in

TABLE 3

The order in which extracts of muscles plus hydrogen peroxide oxidize paraphenylenediamine

NUMBER OF EXPERIMENT	ORDER OF OXIDATION	
1	Di Sar Ext Sol	
2	Di Sar Ext Sol	
3	Di Ext Sol Sar	
4	Di Sar Ext Sol	
5	Di Sar Ext Sol	Same extract as in Exp. 4.
6	Sar Sol Di Ext	Sar. and sol. oxidized simultaneously.

comparison with other mammalian muscles is hardly justified. Moreover, the blackening of the muscle with paraphenylenediamine does not make it necessary even in the mouse to assume a greater actual reserve supply of oxygen. The important fact, which we have shown by our experiments on reduction, is the greater capability of the tissue of the diaphragm to extract oxygen from its immediate environment. Our experiments also make it probable that this peculiarity of the tissue is dependent on the existence in it of an enzyme—it may be extracted from the muscle cells by water and it may be destroyed by heat. Such an enzyme might be a reductase or an oxidase—the existence of either could explain the observed facts—or it might be an enzyme with reversible power. But at present we are not warranted in drawing further or more definite conclusions.

We may, however, be permitted to quote here a few observations that we have made which may possibly bear on the obscure subject of

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peroxidases. Four or five cubic centimeters of the extract of each muscle were poured into a test tube containing an equal quantity of a saturated solution of paraphenylenediamine. Two cubic centimeters of a 0.6 per cent solution of hydrogen peroxide were added in order to give an excess of oxygen as a peroxide, and the tubes were observed for a darkening of the solution. At the end of 2 or 3 hours the colors were finally compared and the results were recorded. The depth of color indicated the degree of oxidation. Six observations were made with the muscles of 5 animals, and the results are given in table 3.

As with reduction, so here the diaphragm ranks first, the soleus last, with the extensor and sartorius between, the latter again leading the extensor.

3. THE METHOD OF STIMULATION

Our experiments in which stimulation was employed have been performed on excised muscles. The animals were killed by decapitation, which occurred instantaneously and painlessly. The muscles were then carefully removed, suspended in moist chambers at room temperature, and attached to light straw levers arranged to record the contractions isotonically. This procedure required usually from 5 to 15 minutes. Thus treated, the muscles of the cat remain irritable and in excellent experimental condition for a considerable time. We can highly recommend such preparations, especially the diaphragm strips, for the general study of muscle phenomena, for demonstration, and for students' use; in various features they are superior to frogs' muscles. The stimuli which we have used were induction currents, usually single break shocks from a Krüger inductorium with the secondary coil placed at 10 cm. In the earliest experiments a single Grove cell was used in the primary circuit. Later this cell was replaced by a small Edison storage battery, and the current in the primary circuit was kept uniformly at 0.7 ampere. The stimuli were maximal in all cases, except where otherwise stated. The electrodes were attached to the two ends of the muscles. In many of our experiments two muscles were used simultaneously for comparison; at such times they were placed in series in the secondary circuit, and the one induction shock stimulated both. The weights lifted were, in most of our experiments, alike for the various muscles, the weight that was usually attached to the lever near its axis being 100 grams, which was equivalent to 10 grams actually lifted by the muscle. In certain experiments weights proportional to the cross-sections of the muscles were employed,

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the calculations being made according to the method of Funaoka (11), namely:

$$\frac{\text{Weight of muscle}}{\text{Specific gravity } (=1.04)} = \text{Volume} \qquad \frac{\text{Volume}}{\text{Length}} = \text{Average cross-section}$$

Computed in this way from 13 specimens of each of the muscles the average cross-sections in square centimeters were as follows: Diaphragm, 0.188; sartorius, 0.192; extensor, 0.275, soleus, 0.349. It was found convenient to use these figures as standard measurements in some of our calculations to be presented later.

4. THE DEGREE OF IRRITABILITY

The relative irritability of the several muscles was measured by determining the threshold of stimulation with the single break induction shock. Only a single muscle from each animal was used and this was

TABLE 4

Irritability of excised muscles as measured by the distance in millimeters of the secondary coil with minimal stimulus from the single break induction shock

	EXTENSOR	SARTORIUS	DIAPHRAGM	SOLEUS
	286	393	313	258
	275	358	424	258
	573	481	357	477
	476	295	314	271
	446	425	368	336
Average.....	411	390	355	320
Percentage.....	100	95	86	78

removed, attached to the recording lever, weighted with a single gram and tested, with the greatest possible speed, so as to insure the least possible diminution of irritability. The average time which elapsed between the death of the animal and the determination of the threshold was 7 minutes. Five experiments were performed with each of the four varieties of muscle and the results are given in table 4.

Although the variations in irritability of each variety of muscle here shown are considerable, the average differences between the different varieties are probably sufficient to be significant. The extensor proved to be the most irritable and the other muscles follow in the order:

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sartorius, diaphragm, soleus. Thus, in the degree of irritability to single induction shocks, the diaphragm lies between the pale muscles on the one hand and the red muscle on the other.

5. THE SINGLE CONTRACTION

The single contractions of the four varieties of muscle differ in certain physical characteristics, as is well shown in figure 1. The diaphragm contracts very quickly to its maximum, and frequently lifts its load to a greater height than do any of the other muscles. The peak of its curve is rather sharp, relaxation beginning promptly. For one third or one half of its course relaxation is quick; it then slows markedly, and



Fig. 1. Curves of the single contraction of four excised muscles of the cat, stimulated by maximal break shocks. The highest curve is that of the diaphragm, the next in height that of the sartorius; the curve of the extensor longus digitorum is low with quick contraction and relaxation, that of the soleus low with slow contraction and relaxation.

from this point onward the return of the lever to the abscissa is very gradual. The sartorius exhibits an equally quick contraction. In the figure its curve is next in height to that of the diaphragm; sometimes it lifts the weight to the greatest height of all the muscles. Relaxation begins slowly and the whole upper half of the curve is much broader than that of the diaphragm; in its latter half the relaxation is still slower but, in general, similar to that of the diaphragm. The curve of the extensor is much like that of the sartorius, but it is on a much smaller scale, so that both contraction and relaxation are completed earlier than with any of the other muscles. The soleus behaves like a typical red muscle; the height of its curve is about equal to that of the extensor, but its contraction is very slow, reaching its maximum only when all the other muscles are well along in their relaxation. Its

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relaxation too is prolonged, although in the final stages less so than the relaxation of the diaphragm.

Besides these physical differences in the character of the single contraction there are differences in the duration of the latent periods. These we have measured in 6 specimens of each variety of muscle. Only a single muscle was taken from each animal, a weight of only 1 gram was employed, and records of from 4 to 8 single contractions with maximal stimulation were quickly made on a rapid drum. An average period of less than 13 minutes elapsed between the death of the animal and the completion of the records. Thirty-five measurements of the latent period were made with the diaphragm and 40 with each of the other muscles. The averages are given in table 5.

TABLE 5

Latent periods of excised muscles in seconds

	EXTENSOR	DIAPHRAGM	SARTORIUS	SOLEUS
	0.00492	0.00861	0.00720	0.00925
	0.00509	0.00561	0.01000	0.01144
	0.00428	0.00738	0.01047	0.00810
	0.00537	0.00794	0.00990	0.00895
	0.00418	0.00734	0.00795	0.00969
	0.00459	0.00780	0.00666	0.00915
Average.....	0.00474	0.00745	0.00869	0.00943
Percentage.....	100	157	183	199

The striking feature here is the fact that the quickly contracting pale extensor exhibits the shortest latent period and the sluggish red soleus the longest, the duration of the two being as 1:2. The stimulated diaphragm gets into action before both the sartorius and the soleus, but not as quickly as the extensor.

6. THE WORKING POWER

The diaphragm is far superior to the other muscles in the power to do work. This has been demonstrated in two ways: first, by comparing the total amount of work which the several muscles are capable of doing before becoming exhausted; and, secondly, by comparing the absolute powers of the muscles.

In comparing the total amount of work of the muscles we followed the method outlined on page 454. The excised muscles were stimulated

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usually at the rate of 28 shocks in the minute, the contractions were recorded on a slow drum as a series of successive vertical lines, and the experiments were continued until the muscles were exhausted. Two muscles from the same animal were usually studied simultaneously. The data here reported give the duration of the working period and the total amount of work that was performed. The amount of work was computed from a measurement by a Coradi planimeter of the total area that was covered in the graphic record by the series of contraction curves. The terminal portion of such an experiment is usually long drawn out, since the muscles, after accomplishing their chief task, continue to respond to the stimuli by minute twitches for a considerable time. The amount of work done in this terminal phase is slight, and measurement of its area in the graphic curve was found to introduce considerable error. It was possible, however, to measure down to a point where the recorded curves just ceased to be 1 mm. in height, with an error of not more than 2 per cent. In the interests of accuracy, therefore, each experiment has been regarded as ceasing at such point, both as regards working period and total work accomplished.

Figure 2 shows characteristic records for the four muscles, the later portions of the records being given only in sections. In initial extent of contraction the diaphragm frequently led, and the other muscles usually followed in the order: sartorius, extensor, soleus, the height of the initial curves of the two latter not differing greatly. The sartorius was usually alone in making a few introductory contractions, rapidly diminishing in height; the diaphragm occasionally performed such contractions. The diaphragm and the sartorius usually exhibited a treppe, which was the more pronounced in the diaphragm. A treppe was rarely present in the extensor, and was absent in the soleus. After the maximum of contraction was reached all the muscles exhibited the usual gradual course of fatigue on to exhaustion, although they differed greatly in the duration of their activity. Table 6 gives precise measurements of the duration of the working power and the total amount of work that was performed by the several muscles. These figures are averages of 14 specimens of the diaphragm, 13 of the extensor, 15 of the sartorius, and 14 of the soleus. The records were made in an investigation, by the senior author and Dr. E. L. Scott, of the working power of muscles when the animals were subjected to different atmospheric conditions. The animals were here kept for a period of 6 hours in a chamber constantly supplied with an abundance of fresh air of a fairly uniform temperature, averaging 20.5°C., and

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of a relative humidity of 52 per cent. This was a combination of conditions that were especially favorable to the organism. Directly after removal from the chamber the cats were killed and the muscles

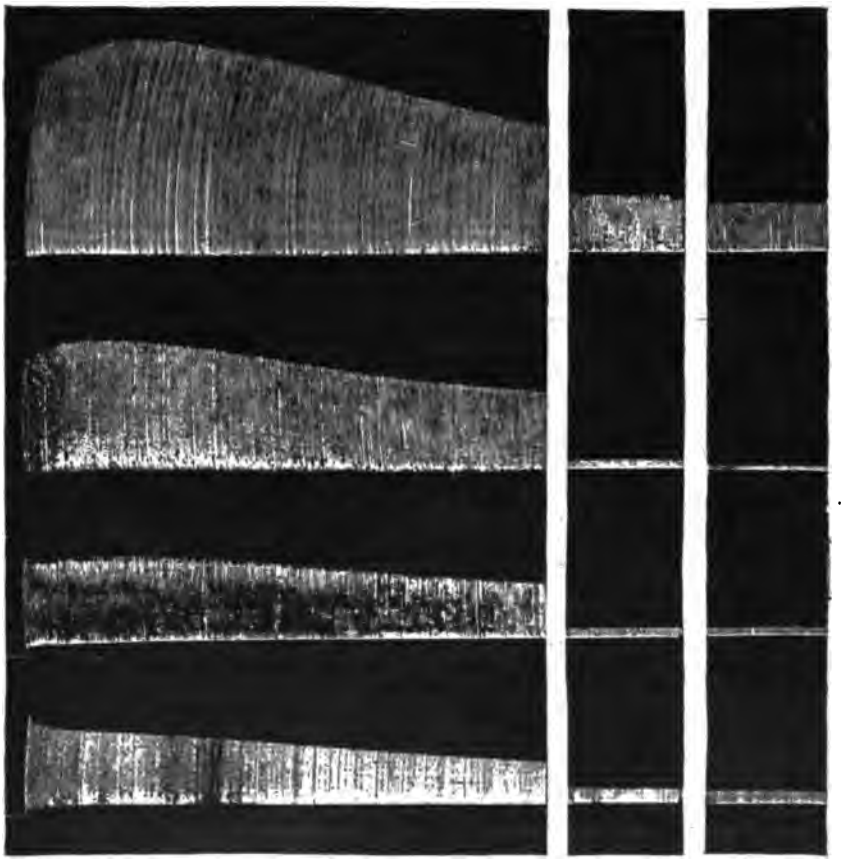


Fig. 2. Curves of series of contractions of four excised muscles of the cat, stimulated by maximal break shocks 28 times per minute and lifting equal loads. From above downward the respective curves are from the diaphragm, the sartorius, the extensor longus digitorum, and the soleus. The selected records belong to corresponding periods of time.

were studied. The details of the experiments will be published elsewhere. In these experiments the actual areas of the cross-sections of the several muscles were not measured, but if we employ the standard

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cross-sections given on page 455 and calculate the total work accomplished per square centimeter of cross-section, we obtain the figures given in table 7.

These tables show that, considered as a machine to do work, the diaphragm is easily the leader of all the other muscles—it works longer and it does more work before becoming exhausted. The long-fibered sartorius can accomplish only about half as much; while the compact short-fibered extensor and soleus perform for each unit of cross-section only one-seventh to one-eighth of the diaphragm's achievement. The latter, in fact, both in the small strip used and in unit of cross-section,

TABLE 6

Average working period and total work of excised muscles before exhaustion; animals subjected to favorable atmospheric conditions

	DIAPHRAGM	SARTORIUS	SOLEUS	EXTENSOR
Working period:				
Minutes	148	121	84	72
Percentage	100	82	57	49
Total work:				
Gram-millimeters	147,957	76,599	31,593	30,224
Percentage	100	52	21	20

TABLE 7

Total work of excised muscles per square centimeter of cross-section before exhaustion; animals subjected to favorable atmospheric conditions

	DIAPHRAGM	SARTORIUS	EXTENSOR	SOLEUS
Gram-millimeters	787,005	398,953	109,905	90,524
Percentage	100	51	14	12

performs much more work than all the other muscles together. In considering this marked difference it should be mentioned that, as we shall see later, the diaphragm survives all the other muscles after death. But this is probably only a minor factor in its power to accomplish much work before exhaustion. Undoubtedly of more importance are its greater ability to utilize oxygen, its greater content in glycogen, which has been determined by Lee, Scott and Colvin and will be discussed by them in a subsequent paper, and perhaps other factors.

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In determining the absolute power of the several muscles we employed the original method of Weber, that is, we determined the maximum load that the afterloaded muscles were just incapable of lifting. We then used the figures thus obtained, together with the standard figures for cross-sections, in computing the absolute power per square centimeter of cross-section. Table 8 shows the results of such determinations in 10 specimens of each muscle.

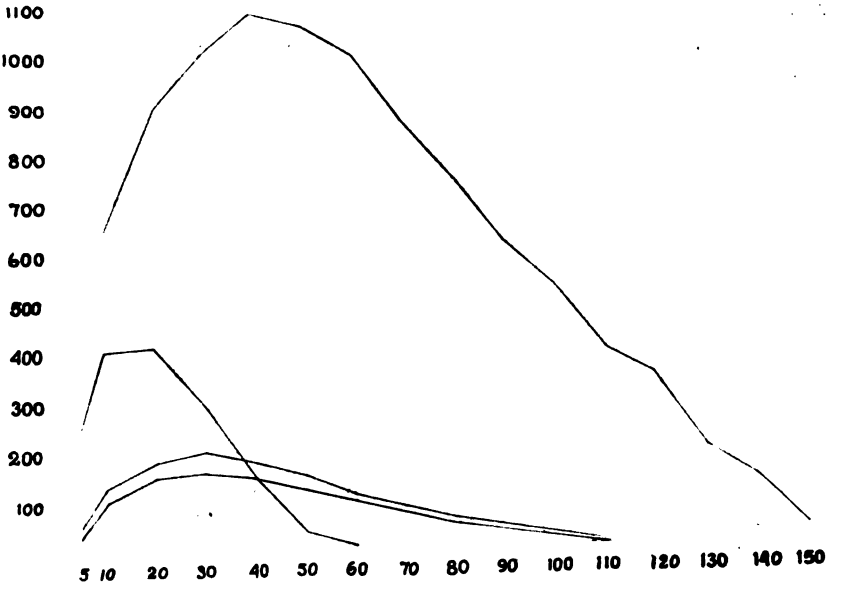


Fig. 3. Composite curves of work of excised muscles of the cat, each curve representing the average of 5 muscles. The order of decreasing height is: diaphragm, sartorius, extensor longus digitorum, and soleus. Abscissa indicates successive grams lifted; ordinates, work done in gram-millimeters per square centimeter of cross-section.

In making the determinations of absolute power it is not a question of survival, for the tests were made quickly after the excision of the muscles; it is simply a question of lifting loads, of doing work. Here the diaphragm is seen again to be far in the lead. It is followed in order by the extensor and soleus, possessed respectively of but three-quarters and one-half of the power of the diaphragm. Last comes the sartorius, relatively weak, with an absolute power of less than one-third.

If the curves of work be plotted for the several increments of load

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beginning with the lightest and proceeding gradually to the heaviest possible and calculating the observed data in terms of unit of cross-sectional area, we have a result as shown in figure 3, which is a composite curve representing the average of 5 observations on each variety of muscle. This reveals, most strikingly of all the various ways of looking at the matter, the supremacy of the diaphragm.

Thus, however viewed, the diaphragm, as a working mechanism, is vastly superior to all the other muscles studied. It lifts a greater load, it is capable of lifting it to a greater height, it continues to work for a longer time, and it performs before it becomes exhausted a much greater amount of labor. All of these powers are greatly to its advantage in the important rôle that it plays in the life of the individual.

TABLE 8

Absolute power of excised muscles, measured in grams per square centimeter of cross-section

	DIAPHRAGM	EXTENSOR	SOLEUS	SARTORIUS
Average	599	458	314	188
Percentage	100	76	52	31

7. SUMMATION OF STIMULI

In the course of many of our earlier experiments on stimulation it became obvious that the four varieties of muscle differed as to their power of summating stimuli. This was finally tested, with the assistance of Mr. C. A. Worth, by the following method. A single muscle was removed from an animal as soon as possible after death, and was prepared for a graphic record. The threshold of irritability for single induction shocks was determined, and the records of a few supraminimal contractions with augmenting stimuli were made. Then the stimulus was increased suddenly to a maximum and a series of contractions was recorded, usually 50 in number at a convenient rate of 28 in the minute. Immediately thereafter the stimuli were reduced to the former supraminimal intensity, and a few contractions were recorded analogous to the first series, but in the reverse order—that is, with stimuli progressively diminishing in intensity back to the threshold. The location of the threshold and the intensity of the contractions following each grade of supraminimal stimulation indicated the degree of the irritability of the muscle at the beginning and at the end of the

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test; the maximal stimuli were employed to augment the irritability, if such were possible, and thus make manifest the power of summation, if such were present. Any augmentation of irritability might readily be explained by the action of metabolites in small quantity, as suggested by the senior author (12) for the treppe. Summation was manifested either by an increase in the extent of supraminimal contraction, or by a lowering of the threshold, or by both together. We have tested in this way 11 diaphragms and 6 of each of the other varieties of muscle. With the diaphragm some degree of summation was always capable of demonstration; with the other muscles it was either absent or showed only very feebly.

The power of a muscle to summate stimuli, that is, the power rapidly to augment responsive contractions upon a slight expenditure of stimulus, is doubtless of advantage to the muscle, and here the diaphragm seems to be superior to the other muscles studied.

8. RHYTHMICITY

The tendency of skeletal muscular tissue to respond by visible twitches, more or less rhythmical in character, to tetanic stimuli much more rapid in rate, or to constant stimuli, such as are afforded by a galvanic current or by solutions of inorganic salts, has received much attention from investigators. One of us (A. E. G. 13) has made, in the sartorius muscle of the frog, a special study of this property and its relation to various ions. In our earlier experiments it appeared to be highly developed in the excised diaphragm, as was readily demonstrated by subjecting the usual strip of the muscle, arranged to record its contractions, to a weak faradic current of from 40 to 200 shocks in the second. As the interrupter in the primary circuit we have used the hammer of the inductorium vibrating at the rate of 48 times in the second, and tuning forks of 20, 50, and 100 v.d. provided with the usual electric platinum-contact device. The responsive contractions were then found to occur usually at a rate of from 1 to 4 in the second, although always somewhat irregular in both frequency and extent, the rate increasing with increasing strength of the stimulating current. If the current were very weak these were the only visible signs of stimulation; but if it were slightly strengthened the muscle might fall into a tetanus, with the twitches superimposed as waves upon the smooth tetanic curve. With still further increase of stimulus the response became still more complex and irregular in both rate and intensity,

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and a single muscle was often found to manifest all degrees of response from the simple to the complex type. All the three other skeletal muscles that we have studied were found to exhibit the same phenomenon, although, as shown by the extent, the frequency, and the regularity, of the individual twitches, the diaphragm led all. The order in

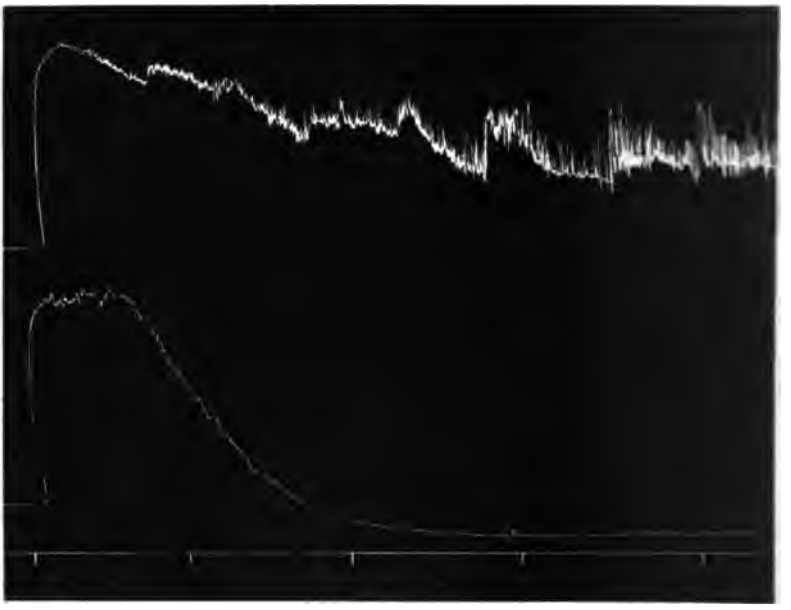


Fig. 4. Curves of contraction of two excised muscles of the cat (upper, diaphragm; lower, sartorius), produced by immersing the muscles in an aqueous solution of equal parts of $\frac{N}{8}$ sodium chloride and $\frac{N}{8}$ sodium oxalate at 35–40°C. Note the greater tendency of the diaphragm to perform "rhythmic" twitches. Time curve records minutes.

which they were arranged as to the degree of such "rhythmicity" was: diaphragm, extensor, sartorius, soleus, the difference between the extensor and the sartorius, however, being slight.

Similar twitches may be readily demonstrated by immersing the muscles—we have tried the diaphragm, the sartorius and the soleus—in solutions of various electrolytes. We have used for this purpose sodium oxalate, sodium tartrate, sodium carbonate, sodium sulphate, and potassium chloride, usually in one-eighth normal solutions and at a temperature of 35–40°C. A very satisfactory solution consists of equal parts of $\frac{N}{8}$ sodium chloride and $\frac{N}{8}$ sodium oxalate. Figure 4

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shows a record obtained with this solution. With all of these substances the diaphragm usually exhibits twitches more numerous and of greater extent than do the other muscles.

In analysing this "rhythmic" tendency as it is demonstrated by the faradic current the first step, naturally, is to exclude any rhythm arising from the stimulating apparatus. For this purpose a method analogous to that of Samojloff (14) has been found convenient. It consists in hanging two strips of the same muscle in series and passing through them the same current, in the same direction, at the same time, and with the same area of contact at the electrodes. Synchronous twitches may then be looked upon as conditioned possibly by an irregularity in the stimulating current. If, on the other hand, the twitches are not synchronous, they may be regarded as conditioned primarily by factors residing in the muscles. We have used this method with the diaphragm only. Certain complications detract somewhat from the clearness and definiteness of the results. Thus, the contractions frequently may differ in height so that in one of the strips they may be barely or not at all evident. As the stimulation is continued, however, the apparently quiescent muscle may begin to show more and more marked contractions while the previously active strip becomes quiescent. Our tests have shown that such variations are not due to changes in the gross relative irritabilities of the two strips. Now the more irritable and now the less irritable preparation may exhibit the more pronounced twitches. But, notwithstanding such differences, most of the twitches appear to be synchronous in the two strips and hence presumably are of instrumental origin.

Samojloff has laid stress upon the imperfections for physiological use of the common methods of interrupting the primary current. Our experience has demonstrated that the presence of a spark gap is fatal to uniformity of successive shocks. Even the introduction of a condenser across such gap in the inductorium, while diminishing the frequency and intensity of the twitches, has failed to eliminate them altogether. Where, therefore, uniformity of successive stimuli is desired, as is especially the case with submaximal stimuli, some device other than the ordinary interrupter with metallic contacts must be found. We have, hence, sought for a different method of stimulation and have found a statisfactory apparatus in a simple alternating current generator consisting of an armature revolving in a field of permanent magnets. For the use of such an apparatus we are indebted to Drs. R. H. Cunningham and H. B. Williams. A photographic record of its

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alternations of potential, made by means of a string galvanometer, shows a series, not of true sine waves, but of waves almost uniform in amplitude and form. The apparatus was set for a speed of 25 cycles, or 50 shocks, in the second and was introduced into the primary circuit of our inductorium. We then obtained with all of our muscles perfectly smooth tetanic curves with no sign of twitches, whatever the intensity of the stimulating current. This experiment seems to prove beyond a doubt that most of the previous "rhythmic" responses to the faradic current originated in quantitative irregularities of the shocks supplied by the interrupter.

But this is not the whole story, for the following striking facts still remain: (1) The diaphragm is much more prone to exhibit such twitches than are the other muscles; (2) there is occasional asynchronism when both of two diaphragm strips are giving well-marked contractions; and (3) the twitches are frequently present in one strip and the other alternately. Furthermore, if it is wholly a question of the irregularities of the stimuli, how are we to explain the similar "rhythmicity" and the similar greater proneness of the diaphragm to it in the presence of electrolytes? Is it possibly a question of differences of polarization with faradic stimuli and differences of permeability with chemical stimuli? Moreover, if we accept Lucas's view that the individual fibers of striated muscle obey the "all-or-none law" and thus reach with our submaximal stimuli only a portion of the fibers in the whole muscle, are we to suppose that now certain groups of fibers are active, and now others, and that differences in the irritability of different groups of fibers play a part in the phenomenon?

A further step in the analysis of the "rhythmicity" might consist in the exclusion of the nerve. This we believe that we have done through the rapid death of the nerve in our mammalian preparations. The phrenic ceases to respond to stimuli within a very few minutes after death and we have no reason to believe that either the intramuscular filaments of the nerve or the motor end-plates continue irritable for a much longer period. Nevertheless, we have been interested in testing the "rhythmic" tendency of muscles after the administration of curare to the animals and then killing them after paralysis has set in. In such cases the responses to both faradic and chemical stimuli are markedly weakened, although they are not eliminated entirely. The curarized muscles are much more prone to pass directly into unbroken tetanic contractions. Has the curare affected the receptive substance of Langley in such a manner as to make the muscle less susceptible to irregularities in the stimuli?

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We thus see that our consideration of the "rhythmicity" of the various muscles, so far as we have carried our experiments at the present time, raises more questions than it answers, and we are not now prepared to propose even an hypothesis with any degree of confidence to account for this great susceptibility of the diaphragm to respond to the ordinary faradic and chemical stimuli by "rhythmic" twitches.

9. THE POWER OF RESISTANCE TO DELETERIOUS INFLUENCES

So far we have considered chiefly those general physiological properties that the several muscles may make use of in their ordinary activities. We have now to turn to their behavior under certain unusual circumstances and especially those conditions that may act unfavorably to the muscles.

a. Fatigue. We may recall here, first, the comparative powers of resistance to extreme fatigue that the muscles show, a power that is measured by the amount of time that elapses before the contracting muscle becomes exhausted and by the total amount of work that it is capable of doing—a power that is far more marked in the diaphragm than in the other muscles.

b. Death of body. A similar difference exists in resistance to death after excision from the body. Here we have made a series of observations with the assistance of Mr. C. A. Worth. The four varieties of muscle excised from the same animal and placed within moist chambers, were weighted with only 2 grams each and were tested at intervals of fifteen minutes as to their power to respond to the stimulus of the usual maximal induction shocks. The duration of survival was measured by the time that elapsed between the moment of decapitation of the animal and the moment of the last visible response of the muscle to stimulation. Table 9 gives the results of ten observations. Compare these with the observations formerly reported by the senior author (15) on the tibialis anticus and the soleus.

This table illustrates the great variations in the power of survival that are exhibited by individual muscles of the same kind. Notwithstanding this the pronounced lead of the diaphragm over the other muscles is obvious. Its long period of survival after death, namely, 9 hours and 44 minutes, is half again as long as that of the red soleus and more than twice as long as that of the pale extensor and sartorius—in other words, the diaphragm is extremely hardy.

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c. Curare. It is often said that the diaphragm is the last muscle of the body to succumb to the action of curare. So far as we have been able to learn, this statement is based on Boehm's (16) experiment of 1895. We have made our own observations on several cats and are able to affirm that the statement is true. Our cats were etherized and tracheotomized for the further administration of ether, and the brachial and sciatic nerves were freed from their surrounding tissues. A few cubic centimeters of a 2.5 per cent solution of curare in physiological salt solution were then injected into either the jugular or the femoral vein, a second dose being given later. The brachial and sciatic nerves were stimulated at intervals with a faradic current and the resulting

TABLE 9

Duration of survival of excised muscles after death, expressed in hours and minutes

NUMBER OF EXPERIMENT	DIAPHRAGM	SOLEUS	EXTENSOR	SARTORIUS
1	16:45	12:25	6:25	5:40
2	6:55	5:10	3:10	3:55
3	3:55	4:40	2:40	2:40
4	11:45	11:00	5:00	3:15
5	4:36	6:51	3:11	7:21
6	10:00	6:20	6:20	2:50
7	8:15	2:15	2:15	8:15
8	12:00		4:15	5:00
9	14:43	6:58	3:43	2:18
10	8:30	5:45	6:15	2:00
Average	9:44	6:49	4:19	4:19
Percentage	100	69	44	44

contractions of the leg muscles were observed. When these muscles ceased to respond to supramaximal indirect stimuli, even though the heart was still beating and maintaining the circulation of the blood, the phrenic nerves were exposed and stimulated by the same strong current and were found still capable of causing the diaphragm to contract. It is hardly necessary to mention that careful precautions were taken against an escape of current. At this stage of the experiment the diaphragm had usually ceased its natural contractions, or they were so weak and irregular as to be incapable of maintaining a sufficient respiration. Meyer and Gottlieb (17) state that "by administering the proper dose [of curare], it is possible to keep a rabbit alive for hours with all its muscles paralyzed except the diaphragm." It is evident that,

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whatever the mechanism—and the mechanism is not entirely evident—the diaphragm is more resistant to the action of curare than are the other skeletal muscles. In this connection it may be mentioned that Calmette (18) states that after poisoning by the bite of the cobra respiration becomes difficult and assumes the diaphragmatic type. This fact should be more fully investigated and its exact significance should be determined.

d. Trichiniasis. It is a known fact that in trichiniasis the diaphragm, together with the intercostal muscles and those of the jaw, the tongue and the eye, is infested with the parasites to a far greater extent than are other skeletal muscles. Rehns (9) correlates this fact with

TABLE 10

Average working period and total work of excised muscles; animals subjected to unfavorable atmospheric conditions

	DIAPHRAGM	SARTORIUS	EXTENSOR
Working period:			
Minutes	142	88	54
Percentage	100	62	38
Percentage loss in working period when compared with table 6	4	27	25
Total work:			
Gram-millimeters	117,317	52,965	23,624
Percentage	100	45	20
Gram-millimeters per square centimeter of cross-section	624,027	275,859	85,905
Percentage	100	44	14
Percentage loss in work when compared with table 6 or 7	21	31	22

the greater supply of oxygen which he assumes to be present in the diaphragm. Stäubli (19) ascribes, without proof, the superabundance of trichinae to a greater blood supply of the muscles in question. Here is further opportunity for investigation.

e. Heat and humidity. In connection with the investigation made in coöperation with Scott and referred to on page 458, we have studied the results of exposing cats to unfavorable atmospheric conditions, and report in table 10 our average figures of the working period and the total work performed. Before the muscles were studied the animals were confined for a period of 6 hours in a well-ventilated chamber, the air of which possessed an average temperature of 32.8°C. and an

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average humidity of 90 per cent, that is, the atmospheric condition was analogous to that of one of the hot and humid summer days in an American city and would have been markedly uncomfortable to a human being. The computations in unit of cross-sectional area were made with the aid of the standard figures given on page 455. Observations were made upon 12 diaphragms, 13 sartorii, and 13 extensors; the soleus was not here studied.

The figures of this table will be appreciated only when they are compared with the corresponding data recorded for animals that had been kept under favorable atmospheric conditions, as presented in tables 6 and 7. It will then be observed that the high temperature and the high humidity to which the animals of the present series were subjected before being tested exercised a deleterious influence on the organism which is expressed in a diminished working period and a diminished total amount of work performed by the muscles. This phenomenon will be discussed in detail and in relation to other data in a report by Lee and Scott. What especially interests us now is the relative effects of the harmful conditions on the three muscles. The working period of the extensor is reduced by 25 per cent and that of the sartorius by slightly more, 27 per cent, while the diaphragm suffers only a 4 per cent reduction. As to working power the sartorius has lost nearly one-third, the extensor and the diaphragm only one-fifth, with the advantage slightly in favor of the latter muscle. Thus, of all the muscles, the diaphragm is least affected by the unfavorable atmospheric conditions.

f. Fasting. In connection with an investigation of some of the physiological effects of inanition, which is being carried on by the senior author together with Morgulis and Scott, the effects of fasting on the diaphragms and the extensors of 10 cats have been studied. The animals were allowed water but no food; the average duration of the fasting period was 15 days; and the average loss in bodily weight was 33 per cent. The details of the work will be published elsewhere, but the average results are given in table 11.

We see here that, as might be expected, there is a great falling-off both in the duration of the working period of the two muscles and in the total amount of work performed. The effect of inanition may be best appreciated when the results are compared with those of the muscles of tables 6 and 7, which were in a good nutritive condition. As regards the working period the diaphragm is again affected less than the extensor, the percentage decrease of the total time being 30 and 40 respectively. As regards the total amount of work that the muscles

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are capable of performing the result is different: the fasting diaphragm has lost 44 per cent of its working power, the fasting extensor only 16 per cent. The respiratory muscle thus suffers to a much greater extent than does the leg muscle. We are not prepared to offer an explanation of this exceptional fact, but we cannot refrain from suspecting that it is associated in some way with the diaphragm's greater activity. The observations on fasting are not yet completed.

TABLE 11

Effect of inanition on the average working period and the total work of excised muscles

	DIAPHRAGM	EXTENSOR
Working period:		
Actual, in minutes	104	43
Percentage	100	41
Percentage loss in working period when compared with table 6	30	40
Total work:		
Actual in gram-millimeters	82,888	25,295
Percentage	100	31
Actual in gram-millimeters per square centimeter of cross-section	440,894	91,982
Percentage	100	21
Percentage loss in total work when compared with table 6 or 7	44	16

10. SUMMARY.

This paper is devoted to a comparative study of several of the general physiological properties of four skeletal muscles of the cat—the diaphragm, the extensor longus digitorum, the sartorius, and the soleus. The general results of the study may be summarized in table 12. Here, after the designation of each physiological property, the degree in which it has been found present in the several muscles is given. The order is from the greatest to the least in all items, except the length of the latent period, in which this order is reversed.

The most common order in which the several muscles may be arranged as to the quantitative development of the various general physiological properties is: diaphragm, sartorius, extensor, soleus. The properties appear least pronounced in the red soleus; and next in the two pale muscles, with the sartorius slightly in the lead. As to most of the properties the diaphragm stands by itself in the lead of all the others.

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Its tissue possesses the greatest reducing power, in other words, its greatest power to remove oxygen from its environment, and this power is probably dependent on the existence of an enzyme; its content of peroxidase is greatest; the irritability of the excised diaphragm to single induction shocks, while surpassed in degree at first by both the extensor and the sartorius, survives long after all the other muscles have died in the brevity of its latent period the excised diaphragm is surpassed by the extensor only; when actively stimulated and made to do work

TABLE 12

Order in which muscles exhibit physiological properties

Reducing power with oxyhaemoglobin	Di	Ext	Sar	Sol
Reducing power with methylene blue	Di	Sar	Ext	Sol
Content of peroxidase	Di	Sar	Ext	Sol
Irritability to single induction shocks	Ext	Sar	Di	Sol
Length of latent period	Ext	Di	Sar	Sol
Working period in cool dry air	Di	Sar	Sol	Ext
Total work per square centimeter of cross-section in cool dry air	Di	Sar	Ext	Sol
Absolute power	Di	Ext	Sol	Sar
Power to summate stimuli	Di	Other muscles		
Tendency to "rhythmicity"	Di	Ext	Sar	Sol
Resistance to fatigue	Di	Sar	Sol	Ext
Duration of survival after death	Di	Sol	[Ext	Sar]
Resistance to curare	Di	Other muscles		
Tendency to trichiniasis	Di	Other muscles		
Resistance to heat and humidity: working period	Di	Sar	Ext	
Resistance to heat and humidity: total work per square centimeter of cross-section	Di	Sar	Ext	
Resistance to inanition: working period	Di	Ext		
Resistance to inanition: total work per square centimeter of cross-section	Ext	Di		

the excised diaphragm will work for a much longer period and accomplish far more than the other muscles—even more than all together—before becoming exhausted; it is superior in absolute power and in tendency to summate stimuli; it exhibits a greater tendency than do the other muscles to respond to faradic and chemical stimuli by twitches, more or less rhythmical in character; its greater resistance to fatigue is exhibited in its longer working period and its greater work accomplished before becoming exhausted; it, with possibly some other respiratory muscles, is the last skeletal muscle of the body to submit to the paralysing action of curare; other investigators have pointed out its greater tendency than other parts of the body to harbor trichinae

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—a peculiarity that is not yet explained, but may possibly be associated with the greater power of the diaphragm to utilize oxygen; when the body is subjected to high heat and high humidity all the muscles (the soleus not having been studied) are affected deleteriously, which is manifested in a diminution of the working period and the total work that can be accomplished before exhaustion sets in, but the diaphragm is affected far less than the other muscles; by extreme inanition the total working period of the excised diaphragm is shortened far less than that of the extensor, although the total work that can be performed by the former is diminished to a greater degree—a fact that may possibly be associated with the greater activity of the diaphragm.

Most of these facts indicate that the diaphragm possesses much more efficient muscular tissue than do the other muscles—in other words, it is a superior physiological mechanism. This is exactly what might be expected, when the unique and superior rôle of the diaphragm among skeletal muscles is considered. Here seems to be a striking instance of physiological adaptation to physiological requirements.

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SOME OF THE CHEMICAL PROPERTIES OF CERTAIN MAMMALIAN MUSCLES

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In the course of the investigation of the general physiological characteristics of certain muscles of the cat, which has been carried on by the senior author together with Guenther and Meleney (1), it proved desirable to know in some detail the chemical constitution of the same muscles and their chemical changes under various experimental procedures. The present paper presents the results of the first portion of this research and consists of a partial chemical analysis of the muscles in question, which are the diaphragm, the extensor longus digitorum, the sartorius, and the soleus. The extensor and the sartorius are pale red in color, and possess in general the physiological properties of pale muscles; the soleus is deep red with the general physiological behavior of dark muscles; while the diaphragm, intensely red in color, cannot be classed physiologically with either of these two classes, but seems in many respects to stand by itself as a superior physiological mechanism. It leads all the other muscles in most of the general physiological properties that have been studied. It is superior in the power of survival after death, in reducing power, in content of peroxidase, in power to work long and to accomplish much before becoming exhausted. in absolute power, in tendency to summate stimuli and to respond to faradic and chemical stimuli by more or less rhythmical twitches, and in resistance to fatigue, to curare, to the depressing action of high heat and humidity, and to inanition. In most of these features the soleus stands at the other end of the series, while the extensor and sartorius occupy an intermediate position, with the sartorius slightly in the lead.

It is obviously desirable to know whether any correlation exists between the physical behavior and the chemical properties of the muscles in question, and a search for such correlation is the main purpose of the present studies. We can hardly pretend yet to have made much advance in this direction. Our present analyses, indeed, should be re-

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garded merely as constituting a reconnaissance, a preliminary survey of a field which appears to be large and not altogether unpromising.

So far the work has been confined to a determination of the chemical elements other than carbon, hydrogen, and oxygen, and of glycogen. In spite of the fact that a distribution analysis would increase the experimental error by the introduction of additional technical procedures and the reduction of the absolute amounts of substances determined, it was thought that the greater physiological significance of the results would warrant the use of this method, which, therefore, we have followed. Since some points have thus been revealed that must have been overlooked by the more direct method, we believe that the results have justified our course. This is especially true in regard to the distribution of the sulphur.

The animals were killed by swift and painless decapitation. The muscles were quickly removed and cleaned so far as possible of external fat, fascia and tendons. From the diaphragm the pleural and peritoneal coverings and the tendinous center were removed. In every case samples of the four muscles were taken from each cat used. The size of the samples (about 30 grams of fresh tissue from 12 to 15 muscles) was determined partly by convenience in extraction and partly by the fact that the material was to a large extent a by-product from other experiments and was collected as opportunity offered. This resulted in the samples being so small that great accuracy in the determination of the inorganic salts could not be expected. Although, in general, the results for the inorganic salts have not been used in our correlation of the chemical and physical properties of the muscles, the analyses were made in the hope that the results would at least point the direction for further and more detailed work; and we believe that this consideration warrants their publication.

The method of separation is that described by Koch (2) and his collaborators. For its details the reader is referred to the original papers. Briefly, the material was collected in a sufficient quantity of redistilled alcohol to make the final concentration of alcohol between 85 and 90 per cent. It was then extracted alternately with alcohol and ether. This operation was carried out in an apparatus so arranged that the actual extraction took place at a temperature but little below that of the boiling solvent.

In this manner the lipins and a certain amount of the water-soluble extractives were separated from the proteins and the remainder of the water extractives. The lipins were then precipitated from the extrac-

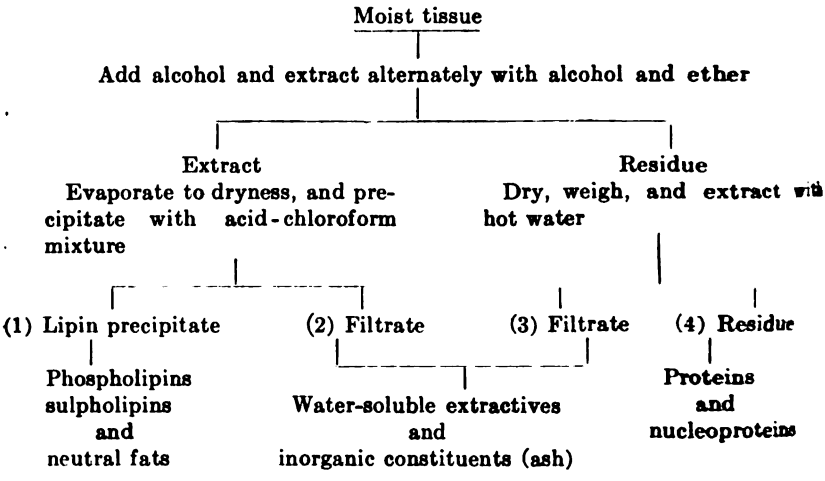
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tives by acidulated chloroform, and the lipin phosphorus and sulphur were determined. The extractive fraction was dried and ashed in hydrochloric and nitric acids, and the iron, magnesium, calcium, potassium, sodium, phosphorus, and sulphur were determined.

The alcohol-ether insoluble portion was extracted repeatedly with boiling water, the ash of the extractives was obtained in the same manner as above, and the same determinations were made.

The nitrogen, phosphorus and sulphur of the protein fraction were determined.

The fractionation may be formulated as follows:



In the present paper it is not thought necessary to describe the details of the methods used in the determination of the several constituents further than to say that the sulphur and phosphorus of fractions 1 and 4 were determined as described by Koch, and the determinations in fractions 2 and 3 were made by the official methods of the Association of Official Agricultural Chemists (3). In the calculations of fractions 2 and 3 corrections were made for the material not removed from the lipin and the protein fractions. In the former case this was done by the method of aliquot parts; in the latter a portion of the protein residue was ashed and the correction was calculated from the ash recovered. In both cases the corrections were small, of the order of 2 per cent.

While in some cases, especially of the inorganic salts, the percentage of variation between the findings in parallel determinations is larger than is desired, it should be remembered that our parallel determi-

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nations were made upon actually different samples, collected at different times, rather than on different portions of a homogeneous sample prepared from a single animal. In this way the variations between samples are added to the technical error. Since, with the one exception discussed below, we have not considered in our interpretation of

TABLE 1

Water, solids, and elementary constituents of muscles in grams per 100 grams of total solids. The figures are the averages found for each of the four muscles studied, together with the averages for those of the series. The figures found by Katz for cat's muscle are given for comparison.

CONSTITUENTS		DIAPHRAGM	EXTENSOR	SARTORIUS	SOLEUS	AVERAGES OF THE SERIES	KATZ'S AVERAGES
Water		73.26	75.15	73.36	75.42	74.14	75.136
Solids		26.74	24.85	26.64	24.58	25.86	24.863
Lipins: Fraction 1	Total weight...	11.73	7.69	11.52	14.13	10.86	
	Phosphorus....	0.1673	0.1803	0.2128	0.1704	0.1835	0.1166
	Sulphur.....	0.0314	0.0424	0.0495	0.0718	0.0469	
Inorganic salts: Fractions 2 and 3	Iron.....	0.0308	0.0235	0.0337	0.0393	0.0309	0.0372
	Calcium.....	0.0356	0.0280	0.0615	0.0474	0.0401	0.0341
	Magnesium....	0.0729	0.0570	0.0773	0.0508	0.0642	0.1152
	Potassium.....	1.5154	1.6412	1.2601	1.6352	1.5251	1.5576
	Sodium.....	0.8751	0.5108	0.5474	0.7229	0.6536	0.2932
	Phosphorus....	0.5042	0.6236	0.5786	0.5380	0.5620	0.6192
	Sulphur.....	0.1872	0.3101	0.2674	0.3251	0.2670	
	Chlorine.....						0.2257
Proteins and nucleoproteins: Fraction 4	Total weight...	69.1	73.4	69.9	68.4	70.5	
	Nitrogen.....	11.159	11.539	11.119	11.214	11.275	
	Phosphorus....	0.1119	0.1449	0.1785	0.0863	0.1267	0.0748
	Sulphur.....	0.5220	0.5286	0.4872	0.4864	0.5088	
Total phosphorus		0.7834	0.9488	0.9699	0.7947	0.8722	0.8106
Total sulphur		0.7406	0.8811	0.8041	0.8833	0.8227	0.8748

results those which varied greatly in this way we have not felt warranted in encumbering this paper with the extended tables that would be necessary to show all the figures. However, the averages for most of the constituents agree rather closely with those of Katz (4), who also studied the muscles of the cat as well as of other animals.

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Tables 1, 2, and 3 show the average results of the determinations of water, solids, and elementary constituents, and table 4 the more detailed results for sodium and potassium. In table 1 the results have been calculated in grams per 100 grams of total solids; in table 2 in grams per 100 grams of fresh tissue; and in table 3 there are given the gram-atoms of the various elements (gram-molecules in the case of

TABLE 2

Elementary constituents of muscles in grams per 100 grams of fresh tissue. The figures are the averages found for each of the four muscles studied, together with the averages for those of the series. The figures found by Katz for cat's muscle are given for comparison.

CONSTITUENTS		DIAPHRAGM	EXTENSOR	SARTORIUS	SOLEUS	AVERAGES OF THE SERIES	KATZ'S AVERAGES
Lipins: Fraction 1	Total weight...	3.137	1.911	3.069	3.47	2.81	
	Phosphorus....	0.0447	0.0448	0.0567	0.0419	0.0475	
	Sulphur.....	0.0084	0.0105	0.0132	0.0176	0.0121	
Inorganic salts: Fractions 2 and 3	Iron.....	0.0082	0.0058	0.0090	0.0097	0.0080	0.0093
	Calcium.....	0.0095	0.0070	0.0164	0.0117	0.0104	0.0085
	Magnesium....	0.0194	0.0142	0.0206	0.0125	0.0166	0.0286
	Potassium.....	0.4052	0.4078	0.3357	0.4019	0.3944	0 3083
	Sodium.....	0.2340	0.1269	0.1458	0.1787	0.1690	0 0729
	Phosphorus....	0.1348	0.1550	0.1541	0.1322	0.1453	0.1539
	Sulphur.....	0.0501	0.0771	0.0712	0.0799	0.0690	
	Chlorine.....						0.5662
Proteins and nucleoproteins: Fraction 4	Total weight...	18.48	18.24	18.621	16.813	18.22	
	Nitrogen.....	2.9839	2.8675	2.962	2.7563	2.9157	
	Phosphorus....	0.0299	0.0360	0.0476	0.0212	0.0328	0.1859
	Sulphur.....	0.1396	0.1314	0.1298	0.1193	0.1316	
Total phosphorus		0.2095	0.2358	0.2584	0.1953	0.2256	0.2016
Total sulphur		0.1980	0.2190	0.2142	0.2171	0.2128	0.2188

water) occurring in 100 grams of fresh tissue. The figures published are the averages of four analyses in the case of the diaphragm, the extensor, and the sartorius, except that only two determinations were made of the inorganic salts of the sartorius. The analysis of only two samples of the soleus was attempted. The averages for the entire series were obtained by adding the amounts of the several elements obtained in the different analyses and dividing the sum by the total

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number of determinations made. In the last column of tables 1 and 2 Katz's figures are given for comparison.

Our figures for sodium are markedly higher than Katz's, being rather more than twice those which he gives for the cat and well above his figures for any animal except the swine and the haddock. It is probable that a certain amount of sodium was extracted from the glass of

TABLE 3

Water and elementary constituents of muscles. The water is expressed in mols, the other constituents in gram-atoms, per 100 grams of fresh tissue. The figures are the averages found for each of the four muscles studied, together with the averages for those of the series.

CONSTITUENTS		DIAPHRAGM	EXTENSOR	SARTORIUS	SOLEUS	AVERAGES OF THE SERIES
Water		4.0665	4.1714	4.0720	4.1864	4.1153
Lipins: Fraction 1	Phosphorus	0.00144	0.00144	0.00183	0.00135	0.00153
	Sulphur	0.00026	0.00033	0.00041	0.00055	0.00038
Inorganic salts: Fractions 2 and 3	Iron	0.00015	0.00010	0.00016	0.00017	0.00014
	Calcium	0.00024	0.00017	0.00041	0.00029	0.00026
	Magnesium	0.00080	0.00058	0.00085	0.00051	0.00068
	Potassium	0.01036	0.01043	0.00859	0.01031	0.01009
	Sodium	0.01017	0.00552	0.00634	0.00773	0.00735
	Phosphorus	0.00434	0.00050	0.00497	0.00426	0.00468
	Sulphur	0.00156	0.00240	0.00222	0.00249	0.00215
Proteins and nucleoproteins: Fraction 4	Nitrogen	0.21192	0.20467	0.21145	0.19674	0.20818
	Phosphorus	0.00096	0.00104	0.00153	0.00068	0.00106
	Sulphur	0.00435	0.00410	0.00405	0.00373	0.00410
Total phosphorus		0.00675	0.00760	0.00832	0.00566	0.00727
Total sulphur		0.00618	0.00683	0.00668	0.00677	0.00663

the apparatus, and at first we were inclined to think that this was the whole explanation of the large amounts. But it will be noted that our figures for potassium are not very different from those of Katz for cat's muscle. Now, by reference to table 4, it will be seen that with the exception of experiment 12 with the extensor, the potassium-sodium ratio is very nearly a constant for any one kind of muscle; and this is so, re-

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gardless of considerable variations in the actual amounts of the elements present. This fact causes us to believe that a possible error from glass is a minor factor in our results, and leads us to point out the possibility that each kind of muscle may have a characteristic potassium-sodium ratio. What the significance of such a specific ratio is, we are not at present prepared to say. Further work in which special attention is paid to the determination of the alkalies is necessary. It

TABLE 4

Sodium and potassium in muscles

MUSCLES	NUMBER OF EXPERIMENTS	GRAMS PER 100 GRAMS OF TOTAL SOLIDS			GRAMS PER 100 GRAMS OF FRESH TISSUE		
		Potassium	Sodium	K/Na	Potassium	Sodium	K/Na
Diaphragm	1	1.3667	0.8417	1.613	0.3660	0.2254	1.624
	4	1.4635	?	?	0.3867	?	?
	9	1.3560	0.8054	1.684	0.3668	0.2179	1.684
	13	1.8755	0.9783	1.917	0.6996	0.2614	1.917
	Average	1.5154	0.8751	1.732	0.4052	0.2340	1.731
Extensor	2	1.4476	0.4852	2.983	0.3518	0.1179	2.984
	5	?	0.5199	?	?	0.1320	?
	8	1.6547	0.6021	2.748	0.4125	0.1501	2.748
	12	1.8214	0.4369	4.169	0.4517	0.1083	4.169
	Average	1.6412	0.5108	3.567	0.4078	0.1269	3.214
Sartorius	7	1.5654	0.6374	2.455	0.4072	0.1658	2.456
	11	0.9547	0.4373	2.183	0.2369	0.1085	2.183
	Average	1.2601	0.5474	2.302	0.3357	0.1458	2.302
Soleus	10	1.4483	0.6241	2.312	0.3600	0.1596	2.257
	14	1.8220	0.8217	2.217	0.4448	0.2006	2.217
	Average	1.6352	0.7229	2.262	0.4019	0.1777	2.262
General average		1.5251	0.6536	2.334	0.3944	0.1690	2.334

is, however, perhaps pardonable to mention the fact that Meigs and Ryan (5) report a potassium-sodium ratio for smooth muscle similar to that which we have found for the diaphragm, while in the cross-striated muscle they find relatively less sodium, as we do in the other three muscles.

In table 5 we have tabulated such of our results with the elementary constituents and such relationships, as seem to be especially significant.

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TABLE 5

Summary of certain elementary constituents of muscles, together with ratios which seem to be significant

CONSTITUENTS	UNITS	ACTUAL QUANTITIES CALCULATED AS INDICATED IN COLUMN 2				PERCENTAGE VALUES, THE FIGURES FOR THE DIAPHRAGM BEING TAKEN AS 100			
		Diaphragm	Extensor	Sartorius	Soleus	Diaphragm	Extensor	Sartorius	Soleus
Lipin phosphorus	Per cent of total phosphorus	21.33	19.00	21.94	21.45	100	89	103	101
Water soluble phosphorus	Per cent of total phosphorus	64.35	65.73	59.64	67.69	100	102	93	105
Protein phosphorus	Per cent of total phosphorus	14.27	15.27	18.42	10.86	100	107	129	76
Lipin sulphur	Per cent of total sulphur	4.24	4.80	6.16	8.11	100	113	145	191
Water soluble sulphur	Per cent of total sulphur	25.30	35.20	33.24	36.80	100	139	131	145
Protein sulphur	Per cent of total sulphur	70.51	60.00	60.60	55.05	100	85	86	78
Ratio of lipin phosphorus to lipin sulphur (P/S)	Gram-atoms per 100 grams of fresh tissue	5.54	4.36	4.46	2.46	100	79	81	35
Ratio of soluble phosphorus to soluble sulphur (P/S)	Gram-atoms per 100 grams of fresh tissue	2.78	2.08	2.24	1.71	100	75	80	61
Ratio of protein nitrogen to protein sulphur (N/S)	Gram-atoms per 100 grams of fresh tissue	48.71	49.93	52.22	52.73	100	102	107	108
Ratio of alkaline earths to alkalies (Ca + Mg)/(Na + K)	Gram-atoms per 100 grams of fresh tissue	5.07	4.70	8.45	4.44	100	93	167	88
Ratio of potassium to sodium (K/Na)	Gram-atoms per 100 grams of fresh tissue	1.02	1.89	1.36	1.33	100	185	133	131

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Koch and Koch (6) find in their work upon rats' brains that, as the tissue becomes older and, therefore, presumably metabolizes less actively, there is an increase in the concentration of colloidal sulphur and phosphorus. Correlated with this we should expect to find less lipin and protein sulphur and phosphorus in those muscles whose physiological properties are the most marked. This is not wholly the case, however, for while the lipin sulphur increases in a ratio that is approximately inverse to that of most of the physiological properties—that is, the diaphragm contains the least, the soleus the most, and the extensor and the sartorius an intermediate amount—the lipin phosphorus, when considered by itself varies without apparent order. But, if one obtains the ratios between lipin phosphorus and lipin sulphur, he is struck by the fact that they stand in even closer relationship to the physiological properties than does the sulphur by itself, although in this case the ratio is direct. Thus it seems that, the more active muscles contain actually less sulpholipin than those less active, while there is in the lipin fraction of the former relatively more phosphorus, when compared with the sulphur, than there is in the latter. A study of the figures published by Koch and Koch reveals the same decrease in the P/S ratio as the tissue becomes older, though they do not seem to have called attention to this relationship. Similar ratios are present, though somewhat less markedly, in the case of the soluble phosphorus and soluble sulphur. This relationship might perhaps be more striking if separate determinations of the neutral and inorganic sulphur had been made.

Our figures for protein sulphur vary directly with both the reducing and the working powers of the three groups of muscles, rather than inversely, as would have been the case had they harmonized with the theory of Koch and Koch. This, however, is very suggestive in the light of the possibility that the cysteine and cystine of the protein molecule are closely related to cellular respiration, as is suggested by Mathews (7). The striking difference between our ratios and those found by Koch and Koch leads one to wonder whether the mechanism of respiration in muscular and in nervous tissues is not as fundamentally different as are their physiological activities.

In view of the very interesting work recently reported by Clowes (8) we are giving the ratios between the alkaline earths and the alkalies (table 5, line 10). So far, however, we have been unable to correlate these ratios with any of the specific physiological properties.

Our figures for glycogen are given in table 6. In the separation of the glycogen from the muscle Pflüger's (9) method was followed, with only

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the modifications made necessary by the small samples used. The central tendon of the diaphragm was rejected, the sample for analysis consisting only of the muscular tissue together with its pleural and peritoneal coverings. In the case of the paired muscles the muscles from both sides were used. Here superficial fascia and tendinous matter were removed, so that the sample represented only muscular tissue so far as was possible. The average weights of the samples were as follows: diaphragm 5.5 grams; extensor 5.2 grams; sartorius 4.2 grams; soleus 5.6 grams. To reduce destruction of the glycogen the muscles were placed in the hot alkali as quickly as possible after removal from the animal and digestion on the steam bath was continued within a few minutes. However, to neutralize any destruction of glycogen that might have taken place during the removal, the muscles were taken out in the definite order: diaphragm, extensor, soleus, sartorius, for the first half of the series, while the order was reversed for the last half of the series. The final determination of sugar was made by Munson and Walker's (10) method; but the small quantities of sugar obtained in some cases required an experimental extension of their table.

TABLE 6

Glycogen in muscles in grams per 100 grams of fresh tissue

NUMBER OF EXPERIMENTS	DIAPHRAGM	EXTENSOR	SARTORIUS	SOLEUS	AVERAGES	REMARKS
1	0.409	0.283	0.431	0.331	0.363	The muscles in experiments 1–5 were removed in the order: diaphragm, extensor, soleus, sartorius; those in the remaining experiments in the reverse order.
2	0.318	0.138	0.081	0.153	0.175	
3	0.469	0.340	0.194	0.244	0.312	
4	0.202	0.287	0.141	0.076	0.177	
5	0.282	0.259	0.126	0.105	0.193	
6	0.179	0.189	0.087	0.031	0.122	
7	0.182	0.045	0.062	0.093	0.095	
8	0.232	0.095	0.088	0.090	0.126	
9	0.245	0.172	0.119	0.102	0.160	
Average.....	0.280	0.201	0.148	0.136	0.191	
Percentage...	100	72	53	48		

As might have been expected with such a shifting substance, the quantities of glycogen in the different specimens vary considerably. The ratios of the averages, however—diaphragm 100, extensor 72, sar-

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torius 53, soleus 48—are sufficiently distinct to be of importance. The two extremes are here again exhibited by the diaphragm and the soleus, the former containing more than twice the amount of glycogen found in the latter. The two pale muscles take an intermediate position with the extensor in the lead. These differences are best interpreted in the light of the following facts.

The reciprocals of Lee, Guenther and Meleney's figures for the reducing power of the muscles, as measured by the percentage number of minutes required to reduce oxyhaemoglobin, are, respectively, diaphragm 100, extensor 94, sartorius 62, soleus 23. These figures and those for the glycogen content, though not actually the same, are alike in forming a descending series with the muscles in the same order. That this general parallelism is not a thing of chance is well shown by comparing the order in which the muscles occur in the several analyses, as shown in table 6.

It is natural to seek correlation also in working power. If we consider this feature, as measured by the total work done by the several excised muscles per square centimeter of cross-section, we find Lee, Guenther and Meleney's figures to be: diaphragm 100, sartorius 51, extensor 14, soleus 12. Here again the diaphragm comes first, the soleus last, and the two pale muscles between, although these two have changed places with one another. This exception to the order is probably of minor significance.

The main feature of our comparison here is that, as regards the amount of glycogen contained in the muscles, their capacity to remove oxygen from their environment, and their capacity to perform work, the diaphragm is always in the lead, the soleus is always last, and the two pale muscles occupy an intermediate position. We cannot help believing that these facts are indicative of a physiological correlation between the three features.

SUMMARY

The present paper contains the results of a determination of the chemical elements other than carbon, hydrogen, and oxygen, and of the glycogen occurring in four muscles of the cat, namely: the diaphragm, the extensor longus digitorum, the sartorius, and the soleus; together with an endeavor to correlate those results with certain of the physiological properties of the same muscles. We have found that:

1. The quantity of lipin sulphur and of water-soluble sulphur is least in the diaphragm, greatest in the soleus, and intermediate in the exten-

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sor and the sartorius; that is, the quantity of non-protein sulphur varies inversely to the order in which most of the general physiological properties of the muscles are expressed.

2. The ratios between the lipin sulphur and the lipin phosphorus and between the water-soluble sulphur and the water-soluble phosphorus vary directly as most of the general physiological properties of the muscles.

3. The protein sulphur varies directly as most of the general physiological properties of the muscles, and is possibly to be correlated with muscular respiration.

4. The glycogen content is greatest in the diaphragm, least in the soleus, and intermediate in the extensor and the sartorius. It thus varies directly as most of the general physiological properties of the muscles, and is to be especially correlated with their reducing power and their working power.

5. For each muscle there seems to be a specific ratio between the potassium and the sodium.

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THE ACTION OF TEMPERATURE AND HUMIDITY ON THE WORKING POWER OF MUSCLES AND ON THE SUGAR OF THE BLOOD

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It is a fact of common experience that a human being in a hot and humid atmosphere feels a disinclination to perform muscular work. If we accept the testimony of various observers, this disinclination is accompanied by actual diminution of working power. Thus, Boycott (1) says that his observations on miners working in hot moist air lead him "to conclude that their power of doing work under these circumstances is quite small." Pembrey's (2) "results show definitely that a man is much less efficient in a warm moist atmosphere." Cadman (3), in his work of mine inspection, says that at 35°C., wet-bulb reading, "work becomes impossible." And others testify to the same effect. There are lacking, however, exact data on which to base these inferences, and, even if they be true, it is not clear whether the impairment of the power to do work lies in the central nervous system, or in the muscles, or in both. It has, indeed, been known since the work of Gad and Heymans (4) in 1890 that the excised muscles of frogs become more rapidly fatigued when warmed, and Patrizi (5) demonstrated in 1893 that human muscles when heated by localized hot baths are subject to early fatigue and exhaustion—and these results may have a bearing on this general topic. The internal conditions of labor under extreme conditions of temperature and humidity have been summarized as follows (6):

> When an individual is subjected to an atmosphere that is charged with an excessively high temperature and high humidity, his bodily temperature is raised, his working power becomes limited, and there is an early oncoming of fatigue. In addition to the normal fatigue substances there are present other substances, products of an abnormal metabolism, perhaps of increased protein disintegration, which likewise act as fatigue substances. Both the normal and the pathological fatigue substances act toxically to diminish the activity of the tissues, and such

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fatiguing action is rendered greater by reason of the abnormally high internal temperature that is present.

In addition to these conditions there are circulatory changes, a drafting of the blood away from the brain and the muscles to the skin, which may aid in the decrease of working power.

Perhaps the effects of extreme atmospheric conditions are different in kind from those of milder conditions, for preliminary reports of the investigations of the New York State Commission on Ventilation (7) appear to show that in an atmosphere of 30°C. (86°F.) and 80 per cent relative humidity there is a disinclination but not an inability on the part of human beings to perform as much muscular work as in a cooler dryer air.

It is evident that analysis of the subject has not yet gone far enough and that the desired data can best be obtained through experiments on animals. It was with a wish to obtain some of these data that the observations here reported were made. In connection with other work we had perfected methods for studying the working power of muscles and for determining the content of sugar in the blood, and we therefore studied these two topics here—not, however, with the preformed idea of showing any necessary relation between them.

Our plan, first, was to compare the effects on these two physiological phenomena of the exposure of animals, on the one hand, to a fairly comfortable atmosphere and, on the other, to an uncomfortably hot and humid one, such as would be afforded by one of the extreme days of a New York summer. This plan was carried out. Later we completed an additional series using an intermediate temperature and humidity.

We used cats as objects of the experiments and subjected them to the desired atmospheric conditions within a box the ventilation of which was controlled. This box was 46 cm. long, 36 cm. wide, and 35 cm. high; its walls were of galvanized iron, and it contained a wooden floor. A cover, clamped on tightly, closed it at the top. An inlet and an outlet tube, each 14 mm. in diameter, at opposite ends of the box, allowed free passage of abundant air, which was drawn through by a suction pump worked by an electric motor and attached to the outlet tube. This ventilation box was set within a larger box, 66 x 56 x 51 cm. in size, which contained water, and thus the air chamber was surrounded on its four sides and bottom by a water jacket, 10 cm. thick. This water was kept at a desired temperature, which varied with the demands of the individual experiment. The course of the air before entering the ventilation box was circuitous and as follows: By the action of the suc-

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tion pump it was taken from the air of the room and passed first into a Williams gas wash-bottle, where it was humidified by being bubbled through water kept at a desired temperature. From this bottle it passed through a lead tube which made eight turns outside the ventilation box within the water jacket, and then entered the box through the inlet tube near the bottom of one end. The outlet tube left the box near the top of the opposite end; from here the outgoing air entered a large glass bottle, which was introduced to protect the pump from excessive moisture, and then passed on to the suction pump, where it was expelled. The cover of the ventilation box contained an observation window and two openings cased with tubing, in which were fixed a dry-bulb and a wet-bulb thermometer. The bulbs of these thermometers projected well into the ventilation box, and the wet bulb with its wick dipping into a small reservoir of water was placed immediately in front of the outlet tube, so that the current of outgoing air swept constantly over the bulb. From the readings of the dry- and wet-bulb instruments the relative percentage of humidity was determined. By carefully controlling the temperature of the water in the water jacket and of that in the Williams bottle, by the use of Bunsen burners or ice, and by the addition of sodium chloride to the water of the wash-bottle in extreme cases where the desired degree of dryness of the air was otherwise difficult to obtain, it was found comparatively easy to control, within the limits desired, both the temperature and the humidity of the air that was supplied to the box. In each experiment a cat was confined within the box for a period of six hours, and records were made every fifteen minutes of the temperature of the air within and of its relative humidity.

At the end of the period of confinement, which was passed usually without excitement and often in a state of repose, the animal was taken quickly from the chamber and killed by swift and painless decapitation. The blood as it flowed from the vessels of the neck was caught in a weighed beaker containing 25 cc. of a 0.5 per cent solution of ammonium oxalate, to prevent coagulation, and was used for the determination of its content of sugar. The muscles that were desired, usually two in number, were excised, and tested for their working power.

The effects of three atmospheric conditions were studied, namely: a comfortable condition, in which the temperature averaged 20.6°C. (69°F.) and the relative humidity 52 per cent; an uncomfortable condition with an average temperature of 32.8°C. (91°F.) and a relative humidity of 90 per cent; and an intermediate condition with an average

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temperature of 23.8°C. (74.8°F.) and a relative humidity of 70 per cent. For convenience we will hereafter designate these three conditions briefly as "low," "high," and "intermediate" respectively.

The two physiological phenomena studied will be considered separately.

THE WORKING POWER OF THE MUSCLES

Three muscles were used, all of which are well adapted to the experimental procedures employed. These were the varieties of muscle that were used by Lee, Guenther and Meleney (8) in their study of the comparative physiological properties of various mammalian muscles, and comprised the sternal strip of the diaphragm, the thin flat medial portion of the sartorius, and the extensor longus digitorum. In addition the soleus was used with the comfortable atmospheric condition. Much previous work had shown that all these muscles are very hardy, will survive and respond to stimulation for long periods of time after removal from the body, and may be treated experimentally in much the same manner as the muscles of a cold-blooded animal. From each animal two of the muscles were carefully excised immediately after death, were placed in moist chambers, were attached to isotonic recording levers, were stimulated in series by the same induction shocks, and wrote their contractions as vertical lines, set close together, on the same slowly moving drum. The stimuli were break shocks delivered 28 times per minute, the current of the primary coil being kept at 0.7 ampere, and the secondary coil being placed at 10 cm. The weight attached to each recording lever was 100 grams, one-tenth of which represented the load actually lifted by the muscle. The records were continued until the muscles were practically exhausted. The total work done was computed in the usual manner after measuring the total area of the graphic record by a Coradi planimeter and calculating from this the total height to which the weight was lifted.

The moment of complete exhaustion of a muscle was found difficult to determine: the later contractions of a series differ in extent from one another almost imperceptibly for a considerable period; and even when the graphic curve becomes an apparently unbroken straight line minute twitches of the muscle may still be seen. It was, however, found impossible to measure accurately the area of the graphic record where the successive lifts of the lever were less than 1 mm. in height. To the point where this height ceased the error in successive measurements

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TABLE 1

The action of a comfortable, or low, temperature and humidity on muscles

NUMBER OF EXPERIMENT	DATE	SEX	BODY WEIGHT IN KG.	AVERAGE DRY BULB TEMPERATURE IN °C.	AVERAGE HUMIDITY IN PERCENT	DIAPHRAGM		SARTORIUS		EXTENSOR		SOLEUS	
						Duration of work in mins.	Work done in gm. mm.	Duration of work in mins.	Work done in gm. mm.	Duration of work in mins.	Work done in gm. mm.	Duration of work in mins.	Work done in gm. mm.
1	Apr. 9	F	2.58	20.0	68	148	81,744			77	31,668		
2	Apr. 20	F	2.70	21.2	66	139	164,456			51	36,589		
3	Apr. 22	M	2.45	21.0	54	118	129,067			15	3,817		
4	June 4	F	3.10	21.1	65	171	206,216			104	46,348		
5	June 5	F	3.08	21.3	54	154	112,324			84	25,334		
6	June 10	F	2.70	21.2	59	166	183,072			85	30,437		
7	June 11	M	3.05	21.8	49	120	167,059			55	25,522		
8	June 15	M	3.75	22.1	50	181	146,992	143	63,725				
9	June 16	F	3.80	21.6	49	180	185,841	56	52,543				
10	June 17	F	2.80	21.4	49	150	183,978	36	19,476				
11	June 25	F	2.25	22.0	49	83	112,474	62	39,756				
12	Nov. 21	F	2.30	20.7	51	154	96,008	29	9,950				
13	Nov. 27	F	3.40	21.2	44	167	178,701	25	8,357				
14	Dec. 5		3.10	21.2	51	145	123,463	200	60,858				
15	Feb. 4	F	3.15	19.7	45			197	193,946	65	36,792		
16	Feb. 6	F	3.05	19.8	45			45	19,620	84	36,375		
17	Feb. 9	F	2.25	19.9	46			151	46,111	66	12,624		
18	Feb. 11	M	2.85	20.1	46			203	207,547	124	40,983		
19	Feb. 13	F	3.65	19.9	48			104	46,126	72	38,365		
20	Feb. 18	M	2.75	19.8	51			254	108,387	59	28,053		

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21	Mar. 9	M	3.01	19.9	58			137	111,422				
								178	161,160				
22	May 13	M	2.96	19.4	52							194	101,742
												73	12,527
23	May 14	F	3.49	20.0	51							127	40,640
												227	70,116
24	May 17	M	3.35	19.6	50							76	27,172
												36	7,455
25	May 18	F	2.98	18.6	50							26	28,392
												30	26,557
26	May 24	M	2.12	20.3	50							16	9,252
												15	7,085
27	May 25	F	2.90	20.4	52							22	11,794
												103	30,601
28	May 26	F	2.78	20.6	57							161	50,775
												72	17,189
Average.....			2.94	20.6	52	148	147,957	121	76,599	72	30,224	84	31,593
Percentage.....						100	100	82	52	49	20	57	21

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was not more than 2 per cent. To insure reasonable accuracy, therefore, each experiment was regarded as terminating at this point.

The results of the experiments with the comfortable atmospheric conditions are presented in table 1.

When we examine these data we observe various points of interest. In the first place, with all the muscles, but especially with the sartorius and the soleus, there is a considerable variation both in the duration of the working period and in the amount of work performed by the several specimens. This is a familiar feature to anyone who has studied the work of muscles; it is not peculiar to warm-blooded muscles—the muscles of frogs vary greatly in these respects. It is obvious from the table that the differences have no relation to body weight, sex, or season. So far as we have been able to judge they have no relation to the animals' general nutritive condition. Moreover, similar muscles from opposite sides of the body of a single individual exhibit the differences—as is well shown in experiments 22, 23, 24, 27, and 28, where the two solei were taken from the same cat. The explanation of the variation is not clear. Rather than rule out the extreme cases, as adventitious abnormalities, we have thought it more just to include all in calculating the averages.

In the second place, all the muscles are capable of continuing to work for a remarkably long time after the first stimulus is received, which is good evidence of their hardiness. Here the diaphragm is far ahead of the others, continuing its contractions for an average of nearly 2 and one-half hours, although the extensor, the first to cease, works for an average of 1 hour and 12 minutes. The sartorius and the soleus are intermediate between these two extremes, the former continuing to contract for 2 hours and 1 minute, the latter 1 hour and 24 minutes.

In the third place, the amounts of work performed by the respective muscles are to be noted, and these constitute for our purposes the most interesting feature of all. Lee, Guenther, and Meleney have found that the average cross-sections of the muscles in square centimeters are: diaphragm 0.188, sartorius 0.192, extensor 0.275, and soleus 0.349. It is a striking fact that the muscle that possesses the smallest cross-section, the diaphragm, accomplishes nearly twice as much work as the sartorius and five times as much as either the extensor or the soleus. As a working mechanism the diaphragm is far superior to the other muscles—a fact which has already been discussed by the former authors and need not detain us here.

We turn next to the action of an atmosphere which possesses a dis-

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tinctly high temperature and high humidity and is markedly uncomfortable to a human being. Our results are presented in table 2.

TABLE 2

The action of an uncomfortable, or high, temperature and humidity on muscles

NO. OF EXP.	DATE	SEX	BODY WT. IN KG.	AV. DRY-BULB TEMP. IN °C.	AV. HUMIDITY IN PER CENT	DIAPHRAGM		SARTORIUS		EXTENSOR	
						Duration of work in mins.	Work done in gm. mm.	Duration of work in mins.	Work done in gm. mm.	Duration of work in mins.	Work done in gm. mm.
1	Feb. 9	F	3.65	30.7	89	138	89,320			82	24,285
2	Feb. 12	F	1.62	33.1	90	61	42,759			8	2,765
3	Feb. 16	F	3.78	35.0	94	200	150,750			51	25,019
4	Feb. 25	F	2.90	32.9	90	195	110,964			35	15,859
5	Apr. 7	F	3.50	33.7	92	114	111,802			65	31,046
6	June 18	F	2.17	32.5	89	193	166,380	33	18,300		
7	June 19	M	3.85	32.9	89	160	130,330	134	181,385		
8	June 22	F	2.75	32.9	89	106	133,263	32	23,982		
9	June 23	M	3.20	33.2	88	133	170,017	131	32,996		
10	June 24	M	2.50	33.6	89	116	126,112	78	35,274		
11	Dec. 12	M	3.40	32.0	90	130	90,224	13	2,383		
12	Dec. 15	M	2.80	32.4	87			176	74,975	60	26,587
13	Dec. 17	F	3.10	32.6	91			112	50,266	38	19,154
14	Jan. 12	M	3.01	32.2	96	161	85,885			28	13,647
15	Jan. 27	F	3.70	32.6	89			155	117,850	18	6,329
16	Jan. 28	F	3.50	32.8	95			48	38,029	57	18,734
17	Feb. 2	F	2.50	32.8	90			36	13,562	63	22,276
18	Mar. 6	M	3.00	32.7	90			36	14,530		
								161	85,008		
19	Apr. 1	M	3.08	32.5	90					92	48,030
										100	53,376
Average.....			3.05	32.8	90	142	117,317	88	52,965	54	23,624
Percentage.....						100	100	62	45	38	20
Percentage change when compared with table 1.....						−4	−21	−27	−31	−25	−22

Here we have a temperature 12.2° (22°F.) above that of the comfortable condition, and a relative humidity 38 points higher. It is a condition that would be debilitating to human beings, and not without the danger of causing heat-stroke if one were exposed to it for hours and at the same time were obliged to perform physical work. The soleus

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was not here studied. The three other muscles again show much variability, in both the duration of work and the total amount of work accomplished; but a computation of the average deviations from the arithmetical average of the total amount of work performed under

TABLE 3

The action of an intermediate temperature and humidity on muscles

NO. OF EXP.	DATE	SEX	BODY WT. IN KG.	AV. DRY-BULB TEMP. IN °C.	AV. HUMIDITY IN PER CENT	DIAPHRAGM		SARTORIUS		EXTENSOR	
						Duration of work in mins.	Work done in gm. mm.	Duration of work in mins.	Work done in gm. mm.	Duration of work in mins.	Work done in gm. mm.
1	Sept. 26	M	2.72	23.8	73	71	33,304			70	21,434
2	Oct. 3	F	3.00	24.1	71	130	152,606			99	46,316
3	Oct. 10	M	3.80	23.8	72	108	100,336			76	29,082
4	Oct. 15	M	3.30	23.6	69	122	94,604			90	33,257
5	Oct. 17	F	3.30	23.6	70	188	154,431			82	44,739
6	Oct. 22	F	3.00	23.8	70	131	117,192	118	86,879		
7	Oct. 31	F	2.95	23.7	69	178	136,886	47	26,971		
8	Nov. 5	?	2.10	23.6	70	187	116,092	28	7,839		
9	Nov. 7	F	2.25	23.6	70	209	215,734	26	9,089		
10	Nov. 12	F	2.20	23.5	70	205	129,304	181	47,037		
11	Dec. 3	M	3.60	23.6	70	119	87,556			109	42,344
12	Dec. 8	M	2.80	23.7	70	169	153,860	32	10,799		
13	Feb. 20	F	2.09	23.8	70			164	117,279	81	34,976
14	Feb. 23	M	2.04	23.9	70			69	41,022	92	35,698
15	Feb. 25	F	2.05	23.8	70			166	66,028	91	48,071
16	Feb. 27	F	2.07	23 9	70			37	14,588	39	14,051
17	Mar. 4	M	3.00	23 9	70			60	77,223	52	26,930
18	Apr. 6	M	2.96	23.8	70					90	46,973
										91	49,460
19	Apr. 8	F	2.07	23.9	70			161	71,405		
								177	137,038		
Average			2 70	23.8	70	151	124,325	97	54,861	82	36,410
Percentage						100	100	70	48	58	32
Percentage change when compared with table 1						+2	−17	−20	−29	+14	+20

the "low" and the "high" conditions reveals a figure lower by about 20 per cent in the latter case. Whether there is a physiological significance in this diminution of variability is not yet clear. The more interesting features are the average changes in the two phys-

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iological phenomena. In both of them all the muscles have lost. In the duration of its work the diaphragm has lost 4 per cent, the sartorius 27 per cent, and the extensor 25 per cent; in the total amount of work performed before exhaustion the diaphragm has lost 21 per cent, the sartorius 31 per cent, and the extensor 22 per cent. It is obvious that the high heat and humidity have profoundly affected the muscular tissue so as materially to diminish its effectiveness as a machine.

Let us examine next the action of an atmospheric environment that is intermediate, in respect to the two features studied, between the comfortable and the uncomfortable conditions. The results are presented in table 3.

Here the average temperature was 23.8°C. (74.8°F.) and the average humidity 70 per cent, a combination of the two features which is not ideal, but would not seem markedly unpleasant to a human being or positively bad. With the three muscles the average degree of variability of the two phenomena studied is essentially the same as in the intermediate condition. In the average duration of the working period, when compared with the comfortable condition, the diaphragm is practically unaffected, the sartorious shows a loss of 20 per cent, and the extensor an actual gain of 14 per cent. In the average total amount of work performed both the diaphragm and the sartorius have distinctly lost—17 and 29 per cent respectively—while the extensor is again exceptional in showing a gain of 20 per cent. Thus, on the whole, the effect of exposure of the animal to the intermediate degree of temperature and humidity is a loss in muscular efficiency, the extent of the loss, however, being less than that which results from the more extreme atmospheric condition.

The above results may be surveyed most clearly when they are summarized as in table 4.

It is here seen that as the temperature and the humidity to which the animals are exposed rise, there is a progressive diminution in both the working period of the muscles and the total amount of work which they are capable of performing after excision. The rate at which the working period falls off—low 100, intermediate 97, high 89—is less than that of the work performed—low 100, intermediate 85, high 76—and the latter feature is probably the more significant of the two. It doubtless indicates that metabolic changes are proceeding, which are of such a character that they react deleteriously on the muscle tissue. That the conditions of metabolism are changed is indicated by the results of our determinations of the sugar of the

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blood. Without doubt, too, the bodily temperature of our animals was raised when the external temperature and humidity rose—abundant evidence has shown that this occurs in human beings under analogous conditions. But we can not now present specific evidence of changes in metabolism under the conditions of our experiments.

It was pointed out in the introduction to the present paper that a human being in a hot and humid atmosphere feels a disinclination to perform muscular work. Our results seem to indicate that this disinclination rests upon a greater physiological basis than a cerebral condition only, whether this be merely a relative cerebral anaemia or an additional depression of cerebral activity through toxic metabolic products. Besides an effect on the nervous system the capacities

TABLE 4

The action of temperature and humidity on muscles summarized. Duration of working period is expressed in minutes; total work performed in gram-millimeters

MUSCLE		TEMPERATURE AND HUMIDITY					
		Low		Intermediate		High	
		Duration	Work	Duration	Work	Duration	Work
Diaphragm	Average	148	147,957	151	124,325	142	117,317
	Percentage	100	100	102	84	96	79
Sartorius	Average	121	76,599	97	54,861	88	52,965
	Percentage	100	100	80	72	73	69
Extensor	Average	72	30,224	82	36,410	54	23,624
	Percentage	100	100	114	120	75	78
Average		113	84,927	110	71,865	101	64,635
Percentage		100	100	97	85	89	76

of the muscles themselves are diminished. Hence excessive muscular work, a whipping-up of the muscles, would tend toward earlier muscular exhaustion, and thus we have additional physiological justification for maintaining that with human beings who are obliged to labor in an atmosphere of extreme heat and humidity excessive and continuous muscular work should be avoided.

THE CONCENTRATION OF SUGAR IN THE BLOOD

As was indicated earlier in this paper, we had no reason to suspect that there is a necessary relationship between the working power of excised muscles and the concentration of the sugar in the blood.

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Indeed, this portion of the work was begun as a continuation of the work previously reported by one of us (9) concerning the effect of common laboratory procedures upon the concentration of blood sugar. But the results seem to correlate very well with those obtained from a physical study of the muscles and hence to have a physiological significance.

The technique of conditioning and handling the animals and of collecting the samples of blood, and the method of analysis were the same as were described in the previous paper. The freedom with which the blood flows from the cut vessels of the neck varies considerably. In our experiments its collection was continued until the free flow ceased, and in practice it was found possible to determine this moment with approximate uniformity. The work of Scott, together with other data still unpublished, shows definitely that, at least when blood is collected from the cat by decapitation, the concentration of sugar decreases as the amount of blood drawn increases, so that the amount of blood drawn must be taken into consideration in the interpretation of results. In doing this 30 grams of blood per kilogram of body weight were taken as the standard and the calculation was made by the formula previously published. This formula is $y=b(x'-x)/a+y'$, in which a and b are constants and equal, respectively, 133 and 0.084, and x' represents the amount of blood drawn, y' its concentration of sugar in any one experiment, x the standard amount of blood per kilogram of body weight, and y the corresponding concentration of sugar.

Our results are presented in table 5. The dates of the experiments and the sex of the animals may be learned from the previous tables.

The first group of figures consists of the results obtained from the control series and show that simple confinement in the chamber is practically without effect when the series is considered as a whole. Thus, the averages for the actual and the calculated concentration of sugar are practically identical and vary but little from those of the standard series, 0.069 per cent, reported in the previous paper. It will be noted, however, that there is much more variation between the individuals of this series than was found for the standard animals. This of course necessitates a longer series than would otherwise be required if the conclusions drawn from such experiments are to be trusted.

Turning next to the last group of figures in table 5, we find among the results, after they have been calculated to 30 grams of blood per kilogram of body weight, but two in the series of twelve which exceed

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TABLE 5

The action of temperature and humidity on the concentration of the sugar of the blood

NUMBER OF EXPERIMENT	NUMBER OF BLOOD SUGAR DETERMINATION	AVERAGE DRY BULB TEMPERATURE IN °C.	AVERAGE HUMIDITY IN PER CENT	BODY WEIGHT IN KG.	AMOUNT OF BLOOD DRAWN IN GRAMS PER KG. OF BODY WEIGHT	GRAMS OF GLUCOSE PER 100 GRAMS OF BLOOD		VARIATION FROM STANDARD OF 0.060 IN PER CENT OF STANDARD	
						Actual	Calculated for 20 gms. of blood per kg. of body weight	From actual concentration	From calculated concentration
Low temperature and humidity									
1	116	20.0	68	2.58	27.6	0.053	0.051	−23	−26
2	117	21.2	66	2.70	33.6	0.079	0.082	+15	+19
3	119	21.0	54	2.45	30.5	0.085	0.085	+23	+23
4	120	21.1	65	3.10	30.1	0.064	0.064	− 7	− 7
6	122	21.2	59	2.70	32.1	0.075	0.076	+ 9	+10
7	123	21.8	49	3.05	27.0	0.052	0.050	−25	−28
8	124	22.1	50	3.75	31.8	0.073	0.074	+ 6	+ 7
9	125	21.6	49	3.80	29.4	0.067	0.067	− 3	− 3
10	126	21.4	49	2.80	21.1	0.063	0.057	− 9	−17
Average		21.3	57	2.98	29.2	0.0679	0.0673	− 2	− 2
Percentage					100		100		
Intermediate temperature and humidity									
6	141	23.8	70	3.00	29.2	0.067	0.065	− 3	− 6
8	145	23.6	70	2.10	34.9	0.079	0.082	+15	+19
10	146	23.5	70	2.20	26.3	0.053	0.051	−23	−26
11	147	23.6	70	3.60	26.7	0.069	0.067	± 0	− 3
12	148	23.7	70	2.80	35.8	0.061	0.065	−12	− 6
Average		23.6	70	2.74	30.6	0.066	0.066	−4	−4
Percentage					105		98		
High temperature and humidity									
1	111	30.7	89	3.65	22.8	0.053	0.047	−23	−32
2	112	33.1	90	1.62	34.3	0.064	0.067	− 3	−17
3	113	35.0	94	3.78	18.5	0.065	0.058	− 6	−16
4	114	32.9	90	2.90	22.5	0.054	0.049	−22	−29
5	115	33.7	92	3.50	23.8	0.062	0.058	−10	−16
6	127	32.5	89	2.17	39.1	0.080	0.086	+16	+25
11	149	32.0	90	3.40	27.9	0.070	0.068	+ 1	− 1
12	150	32.4	87	2.80	24.6	0.061	0.058	− 1	−16
13	151	32.6	91	3.10	24.4	0.071	0.067	+ 3	− 3
14	152	32.2	96	3.01	32.1	0.060	0.061	−13	−12
15	153	32.6	89	3.70	27.4	0.078	0.076	+13	+10
16	154	32.8	95	3.50	25.3	0.062	0.059	−10	−14
Average		32.7	91	3.09	26.9	0.065	0.063	−6	−9
Percentage					92		94		

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the standard of 0.069 per cent. And of these two one (Exp. 6), is so large that it leads one to suspect that some unrecognized factor has been imposed upon the animal. But, in spite of this, the average for the series 0.063, is well below that of the standard, and this fact, together with a tendency toward a low concentration of sugar, leads us to believe that this represents the typical condition of affairs when the animal is exposed to combined high temperature and high humidity. It will be noted also that the quantity of blood obtained is less—by 8 per cent—for these animals than for those of the control series. Whether or not this is indicative of a lessened vigor of the circulatory musculature, more or less similar to that shown for the skeletal muscles, or whether, as seems more probable, it is related to the cutaneous vasodilation that may be presumed to occur under the conditions to which the animals were subjected, we are not at present prepared to say.

Again, because the series is too short to warrant such conclusions, we are not prepared to attach significance to the intermediate concentration of sugar found in the blood of animals subjected to the intermediate conditions, as shown by the second group of figures in table 5. It is interesting to note, however, that the results as they stand are in exact harmony with those of the other two series on the basis of our interpretation.

We desire to call attention to the fact that we recognize that the depression of the concentration of blood sugar found by us is not great; in fact, it may possibly be considered by some persons as not beyond the range of experimental error. We believe, however, that such a contention is unjustified. In demanding extreme variations before significance is attached to them the physiologist must of necessity shut his eyes to many fundamental changes in physiological conditions. And, further, we would emphasize the fact that a lowering of the concentration of blood sugar is much more safely attributed to a given set of experimental conditions than is an increase of the concentration. This is because, as has been abundantly shown, many common factors cause a marked increase in the sugar, while comparatively few have been demonstrated to decrease it.

Any attempt to show the nature of the physiological significance of the lowered concentration of sugar found in animals exposed to conditions of high temperature and high humidity leads only into hypothesis, since many necessary data are not available. But it may not be amiss to call attention to the idea advanced by Cannon (10)

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that the increased concentration of sugar in the blood of animals during intense emotions, together with the mechanism by which the increase is brought about, may be considered as a physiological adaptation. According to this idea the increased amount of sugar that frequently is present in the blood during an emotion is for the purpose of supplying the muscles with the increased energy required for the physical activity necessitated by the emotion. If there is, as this author thinks, a physiological adaptation of the fuel supplied to the increased amount of work to be done, it seems to us not unreasonable to believe that the reverse may also be true. In other words, when it is physiologically fitting that the animal reduce muscular exertion to a minimum, in order that the output of heat may be as low as possible, as in a hot and humid environment, the supply of fuel will be lowered correspondingly.

The complete proof of the hypothesis would require a calorimetric study of animals subjected to these conditions and, perhaps, especially a determination of respiratory quotients. It would also be of interest, in view of current theories of the mechanism of the mobilization of sugar, to determine the amount of adrenalin in the blood of these animals as compared with that of normal animals.

SUMMARY

Cats have been exposed for periods of six hours to atmospheric conditions varying only in respect to their temperature and their humidity. Three conditions have been studied, namely: a "low" condition, in which the average temperature was approximately 21°C. (69°F.) and the average humidity 52 per cent; an "intermediate" condition, in which the average temperature was 24°C. (75°F.) and the average humidity 70 per cent; and a "high" condition, in which the average temperature was 33°C. (91°F.) and the average humidity 90 per cent.

Muscles taken from the animals immediately after such exposure and stimulated to exhaustion show that the average duration of their working periods and the average total amounts of work performed decrease progressively in the three groups from the low, through the intermediate, to the high condition. Expressed in percentages the figures for the duration of working periods are: low 100, intermediate 97, high 89; those for the total amounts of work performed are: low 100, intermediate 85, high 76.

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The degree of variability in the total amount of work performed by the muscles is less under the influence of the intermediate and the high atmospheric conditions, than under the low condition.

The amount of blood per kilogram of body weight that flows from the severed neck vessels on decapitation of animals subjected to similar exposures is less after the high than after the low condition. The percentage figures are: low 100, high 92.

The concentration of sugar in the blood of such animals decreases progressively in the three groups from the low, through the intermediate, to the high condition. The percentage figures are: low 100, intermediate 98, high 94.

It has been shown that under proper precautions cats confined in a small, well ventilated chamber may legitimately be used for experiments involving the determination of the sugar in the blood.

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(5) Patrizi: Arch. ital. de. biol., 1893, xix, 105.
(6) Lee: Amer. Journ. of Public Health, 1912, ii, 863.
(7) Winslow, Kimball, Lee, Miller, Phelps, Thorndike, and Palmer: Amer. Journ. of Public Health, 1915, v, 85.
(8) Lee, Guenther and Meleney: This Journal, 1916, xl, 433.
(9) Scott: This Journal, 1914, xxxiv, 271.
(10) Cannon: This Journal, 1914, xxxiii, 357; Bodily changes in pain, hunger, fear and rage, New York, 1915.

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THE EFFECT OF ETHER ANAESTHESIA ON THE ELECTRICAL ACTIVITY OF NERVE

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From the Laboratory of Physiology in the Harvard Medical School

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INTRODUCTION

The experiments herein described were performed as a preliminary control with reference to an investigation now in progress in this laboratory on the effect of ether anaesthesia on the afferent impulses in the brain stem.[2] At Dr. Cannon's suggestion it was planned to determine by the recording of action currents with the string galvanometer, whether general surgical anaesthesia blocks the afferent impulses arising from peripheral stimulation at the synapses (or cell bodies) through which they must pass to reach the cerebrum and cerebellum. In view of the uncertainty which still exists as to the question whether the electrical disturbance is an inevitable accompaniment of the nerve impulse, it was deemed necessary, first, to determine whether by any treatment with ether it was possible to abolish the action current while still the nerve could be shown by other methods to be capable of conducting a nerve impulse. If this were the case, it is evident that abolition of action currents by ether anaesthesia would not prove abolition of nerve impulses.

We were led by an observation made by one of us in another series of experiments and reported with Gregg[3] to suspect that profound general anaesthesia might so affect a nerve trunk as to abolish the action

[1] We wish to thank Mr. M. Fremont-Smith for assistance in the first two experiments.

[2] A preliminary report on this investigation has already been made; see Forbes and Miller: This Journal, 1916, xl, 148 (proceedings).

[3] Forbes and Gregg: This Journal, 1915, xxxix, 194.

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current. On this occasion a cat was subjected to extremely profound etherization during the operation of decerebration. Immediately after the completion of this procedure, the peroneal nerve was dissected out and connected with the galvanometer. On stimulating the nerve we failed to detect any action current. The nerve was then immersed for over an hour in mammalian Ringer solution at room temperature. It was then connected again with the galvanometer and this time yielded action currents on stimulation. This led us to suppose that the previous absence of electrical disturbance might in some way have resulted from the high concentration of ether in the blood, and that subsequent immersion in Ringer solution might have caused the elimination of ether and consequent restoration of a normal electrical condition. In view of our subsequent experimental results (to be here described) we are led to believe that the absence of action currents in this case was due to some accident in technique or experimental condition which was not noted, or to some other obscure cause not necessarily related to the use of ether.

In reviewing the literature we are concerned with facts bearing (1) on the question of the effects of ether upon nerve trunk activity in general and (2) on the question of the separability of action current and nerve impulse under any conditions. In regard to the first question, Cushny[4] states that nerves are not affected by ether when inhaled, but he cites Waller as having shown that when a frog's nerve is exposed to ether vapor in weak dilution, "its irritability is at first increased," while strong vapor temporarily abolishes the excitability. Borrutau[5] has investigated the effect of narcotics on the action current in frog's nerve as recorded with the capillary electrometer. He reports that alcohol, ether, chloroform and cocain produce no pronounced lengthening of the duration of electrical negativity; though in the course of their depressing effect there is retardation of conduction and delay in the subsidence of the action current. His electrometer records illustrate the effect of ether narcosis, a reduction of the action current occurring after five minutes and complete abolition after ten minutes; subsequent restoration occurring fifteen minutes after the withdrawal of ether.

In regard to the second question, the separability of action current and nerve impulse, Gotch[6] has argued that the nerve impulse may in

[4] Cushny: Textbook of Pharm. and Therap., 1906, p. 163.

[5] Borruttau: Pflüger's Arch., 1901, lxxxiv, 350.

[6] Gotch: Journ. Physiol., 1902, xxviii, 51, etc.

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certain cases occur without electrical concomitant. Other investigators have discussed the same question, including Wedensky[7] and Borruttau[8] who have contended that the electrical disturbance is inseparable from the nerve impulse. The arguments on both sides have been reviewed by Lucas[9] and the conclusions more recently summarized by Forbes and Gregg.[10] It will suffice to state here that all efforts to prove that either the nerve impulse or the electrical disturbance which normally accompanies it, can occur without the other, have failed. The preponderance of evidence so far accumulated supports the view that the electrical disturbance is an inevitable adjunct of functional activity.

One more fact in regard to the estimation of functional activity in the nerve trunk must be noted before proceeding to the description of our experiments. Meltzer and Auer[11] found that ether inhalation in the dog reduces the height of contraction of skeletal muscle in response to stimuli applied directly or to the motor nerve. The muscle no longer responds with tetanic contraction to rapidly repeated stimulation of the nerve, and shows evidence of increased fatigue. The authors infer that ether exerts an action on the motor endings of the nerve similar to that of curare. This result obviously furnishes no evidence as to the effect of the drug on the nerve trunk, inasmuch as nerve fibre and nerve ending are physiologically distinct, but it indicates a factor which must be reckoned with in interpreting observations on the functional activity of the nerve trunk. Muscular contraction following stimulation of the nerve is proof of the occurrence of the nerve impulse, but absence of contraction is no proof of functional inactivity in the nerve.

METHOD

Two methods were adopted in our experiments. One was to determine whether the most profound anaesthesia which could be induced in the cat by inhalation of ether sufficed to abolish or clearly reduce the action current in a nerve trunk directly stimulated; and if it did abolish the action current, whether it simultaneously abolished the contraction in the innervated muscle, this being the only index of functional activity in the nerve. The second procedure, substantially that of Weden-

[7] Wedensky: Pflüger's Arch., 1900, lxxxii, 132.
[8] Borruttau: Loc. cit., 325.
[9] Lucas: Proc. Roy. Soc., 1912, lxxxv, 502-508.
[10] Forbes and Gregg: Loc. cit., 215–217.
[11] Meltzer and Auer: Journ. of Pharm., 1914, v. 521.

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sky,[12] was to expose the nerve trunk of either cat or frog to the direct action of ether vapor and note whether at any time contraction in the innervated muscle persisted when the action current of the nerve was no longer obtainable. Since the muscular contraction was to be used as an index of functional activity in the nerve, it was necessary to record the action currents diphasically; and in order to separate the phases far enough to admit of well defined galvanometric excursions, the leading-off lectrodes were placed as far apart on the nerve as they conveniently could be without bringing the proximal lead near enough the stimulating electrodes to produce confusing "artefacts."[13]

The apparatus, consisting of a Cambridge string galvanometer and photographic recording camera, has been described in detail in a previous paper.[14] In these experiments, as in the last few reported in that paper, an arc lamp was used for illumination, and the large cylindrical lens employed with the Nernst lamp was thus dispensed with. The galvanometer was provided with the low resistance magnet coil excited by eight Edison cells. The platinum string, designated "String C" in the previous paper,[15] was used throughout. The wiring and the stimulating apparatus were exactly as described therein.

When the effect of general anaesthesia on the nerve trunk in the cat was to be studied two procedures were employed. In the first of these the sciatic nerve of the anaesthetized cat was laid bare from hip to knee, but was not at first dissected out from the surrounding tissues, the chief aim being to avoid disturbing its blood supply. For a distance of 2 or 3 cm. at the hip that portion of the sciatic nerve which branches off farther down as the peroneal, and which can be plainly seen as a distinct bundle, was carefully dissected away from the rest of the nerve, ligated and cut at the most central point so dissected. The blood supply to the major part of the nerve between the hip and the knee is not disturbed by this operation.

A pair of non-polarizable boot electrodes was set up in the moist receiving chamber, previously described. A pair of platinum stimulating electrodes was fixed at the opposite end of the chamber from the opening through which the nerve was to be drawn in.

Ether was administered with a bottle through a tracheal cannula: the depth of anaesthesia was gauged by frequent observations of the

[12] Wedensky: Loc. cit., p. 139.
[13] See Forbes and Gregg: Loc. cit., 186, etc.; also fig. 18.
[14] Forbes and Gregg: This Journal, 1915, xxxvii, 121–132.
[15] Loc. cit., 122.

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corneal reflex, the pinna reflex (retraction of the pinna evoked by pinching it with forceps) and the character of respiration. From time to time the separated nerve bundle at the hip was stimulated with a break shock and the resulting contraction of the tibialis anticus muscle noted. With the apparatus in readiness the ether was crowded on until respiration almost or wholly ceased, then the peroneal nerve was quickly dissected from the rest of the sciatic nerve and from the surrounding tissues all the way from the hip to its entrance into the tibialis anticus muscle. This dissection could readily be completed during the time that the animal was too deeply anaesthetized to breathe spontaneously,

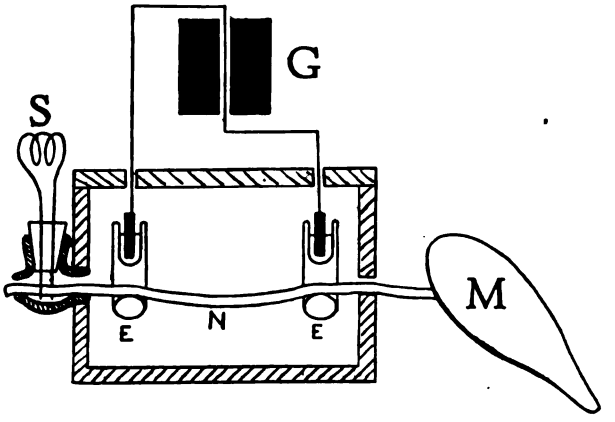


Fig. 1. Arrangement of cat's peroneal nerve in moist chamber for recording action currents. *N*, nerve; *M*, muscle; *S*, stimulating inductorium; *E.E*, "boot" electrodes; *G*, string galvanometer. The details of the galvanometer circuit, omitted here for simplicity, were exactly as shown in figure 1 of the paper cited, this Journal, 1915, xxxvii, 124.

and yet the animal could be revived by artificial respiration and thus maintained for further experimentation. As soon as the peroneal nerve was dissected out it was quickly laid across the non-polarizable electrodes in the moist chamber, and the ligatured central end was connected with the stimulating electrodes. Thus the nerve, having been separated from its blood supply when this was most heavily charged with ether, was arranged as indicated in figure 1, with leading-off electrodes between the point of stimulation and the muscle. Break shocks were then applied and the muscular contractions noted while the string galvanometer was used to detect the presence of diphasic action currents arising from the passage of the impulse over the leads in the moist chamber.

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After this experiment was performed, it was, of course, impossible to use this nerve for a repetition of the same experiment, but it was possible to subject the nerve trunk to the direct action of the ether vapor. But in the two cases in which this was tried the results were unsatisfactory since failure in conduction appeared in the nerves, apparently unrelated to the effects of ether. For this reason our conclusions in regard to the direct application of ether vapor to nerve are based on our experiments with the frog's nerve-muscle preparation which will be described presently.

In the second procedure for the study of general anaesthesia the sciatic nerve in the etherized cat was exposed from the hip to the knee, and the whole nerve cut at the hip as far up as possible. The distal cut end was then ligatured to facilitate manipulation, and dissected from the surrounding tissues far enough to admit of the application of a pair of Sherrington shielded electrodes. The peroneal branch was carefully freed from the surrounding tissues for a sufficient distance between its departure from the popliteal branch and its entrance to the tibialis anticus muscle to permit the application of a boot electrode without contact with other tissues. The tip of one boot electrode was then brought in contact with the sciatic nerve in the thigh region, and the other was applied to the peroneal nerve at the knee. The galvanometer was thus connected with the nerve in a region where the blood supply was undisturbed. Etherization was then crowded to the point of abolishing spontaneous respiration, and frequent records were taken of the action currents resulting from single shocks as the anaesthesia deepened. In one experiment with this method the nerve to the hamstring muscles, branching from the sciatic between the stimulating electrodes and the proximal lead, was not cut, and because the uncut nerves with this arrangement provided an adequate conducting path, the action current of the hamstring muscles entered confusingly into the records. In a subsequent experiment the hamstring nerve was cut and the disturbing factor thus eliminated. It should be noted that with this mode of application of electrodes a shift of contact results from the contraction of the muscles, thus causing an excursion of the string due to change in demarcation current. But this is of no consequence when the action current is recorded on a rapidly moving film, for the latencies of nerve action current and mechanical disturbance are so different as to render it easy to distinguish between them.

In a final experiment the sciatic nerve was exposed but not dissected at all nor disturbed as to its circulation until the animal had

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been etherized to the point at which respiration had ceased and the heart had almost stopped beating. The sciatic nerve was then quickly dissected out, severed at hip and knee, and placed at once on stimulating and leading-off electrodes.

When the second general method was employed, viz., that of exposing a nerve trunk to ether vapor, the frog's nerve-muscle preparation (sciatic-gastrocnemius) was placed in a moist chamber with a pair of boot-electrodes for leads between the stimulating electrodes and the muscle. After one or two diphasic action currents had been recorded, cotton soaked in ether was placed on the floor of the moist chamber under the nerve and between the stimulating electrodes and the proximal lead. It was placed here to insure greater concentration of ether vapor in the neighborhood of the proximal lead than at the neuromuscular junction; for if the action of the ether were to occur at the junction, the muscle might cease to contract before that part of the nerve trunk from which the action current was led had been affected. Records were taken at frequent intervals as the ether acted, and the muscle was watched closely, till contractions ceased. One or two more records were taken after the cessation of contraction, and then the ether was removed and the nerve permitted to recover. The experiment was then repeated. Each preparation studied was etherized in this way twice.

EXPERIMENTAL RESULTS

The results will be described in the same order as the procedures. First, in regard to gauging anaesthesia by reflexes, it was found that the corneal reflex disappeared with a moderate degree of anaesthesia, while the pinna reflex persisted much longer, lasting in almost every case about as long as respiration. There was a somewhat notable uniformity in this approximately simultaneous disappearance of pinna reflex and spontaneous respiration. In all experiments with general anaesthesia it was found that muscular contraction in response to motor nerve stimulation remained vigorous at all depths of anaesthesia, even after respiration had ceased altogether. Therefore, it is clear that the nerve impulse still passes in the motor fibres under these conditions. The next question is whether the action current persists with it. A fairly conclusive answer was found in these experiments in which the peroneal nerve was dissected from its blood supply during profound etherization.

In one of these the nerve was isolated, as etherization was being

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pushed, some time after the corneal reflex had disappeared, but while the pinna reflex was present and respiration only slightly impaired. When the nerve was connected with the galvanometer and stimulated vigorous diphasic action currents were recorded. Thus it is evident that at the somewhat profound surgical anaesthesia here tested the electrical disturbance is not abolished.

In another experiment with this method the peroneal nerve was not dissected from its blood supply till respiration had ceased; the dissection was then performed while artificial respiration was being applied to revive the animal. Figure 2 shows the action currents obtained one minute (*A*) and five and a half minutes (*B*) after the dissection was completed, the dissection taking three and one-half minutes from the cessation of respiration. These action currents are approximately as large as would ordinarily be obtained from the peroneal nerve of a decerebrate cat that has fully recovered from the anaesthetic. It is clear, then, that a nerve cut off from its blood supply at a time when this contains ether in sufficient quantity to abolish respiration, not only performs its physiological function but exhibits a substantially normal action current as well.

Confirmatory evidence was sought by the method described next in order, viz., the recording of the action current from the sciatic nerve whose blood supply remained intact while etherization was pushed to the limit. In the first experiment, that in which the hamstring nerve was not cut, the results were rendered confusing, as already noted, by the entrance of the action current of the hamstring muscles.

In the experiment in which the hamstring muscles were thrown out of action by section of their motor nerve we obtained a most satisfactory confirmation of the conclusion already arrived at by the other method. With electrodes applied as described, one to the sciatic nerve about 2 cm. below the hip, one to the peroneal nerve at the knee, action currents were obtained at all depths of etherization. Figure 3 shows one (*A*) obtained under moderate anaesthesia while respiration was normal, and another (*B*) four minutes later after respiration had ceased.

In the final experiment on general anaesthesia in which a cat was etherized to death, and the sciatic nerve dissected almost at the moment when circulation ceased, the most convincing evidence was obtained. When the nerve was removed from the animal's body and laid across stimulating and leading-off electrodes with a crushed point between the latter, action currents were recorded which compare well with those of the sciatic nerve removed from the decerebrate animal without anaesthesia. The record of one of them is shown in figure 4.

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The results of the experiments in which ether vapor was applied directly to the exposed frog's nerve were also uniformly clear in their bearing on the question at hand. As the contractions grew weaker the action currents grew smaller till finally both were abolished, but in every instance contraction disappeared first. In one instance records were taken showing the persistence of the action current after contractions had ceased, and, when the ether had been withdrawn from the chamber, the presence of a well defined action current a few seconds

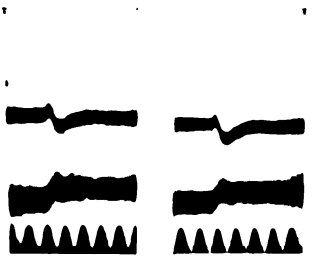


Fig. 2

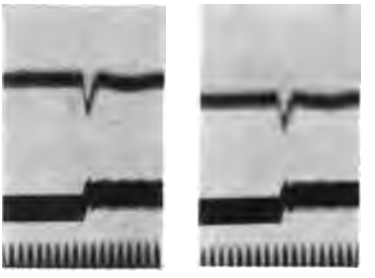


Fig. 3



Fig. 4

Fig. 2. Experiment 5. Diphasic action currents in cat's peroneal nerve; see text. In this and all other photographic records the top line shows the excursions of the string. The second line shows the time of stimulation; a rise in this line shows the break of the primary current. The small oscillations following the break are vibratory and do not indicate secondary closure of the circuit. The bottom line records time; each complete vibration = 0.01 second. In all except figure 3 upward excursion of the string means fall of potential in proximal lead.

Fig. 3. Experiment 7. Action currents in cat's sciatic nerve; see text. In this experiment the lead wires to the galvanometer were reversed, thus causing the string to move down instead of up.

Fig. 4. Experiment 8. Monophasic action current of cat's sciatic nerve; see text.

after contraction was first seen (fig. 5 *A*). In other cases the action current remained appreciable, though very small, in several successive records taken after contraction had ceased (fig. 5, *B*). In no instance was contraction seen when the action current was absent.

The fact that action currents are to be found in the nerve after contraction had ceased does not, of course, prove any lack of parallelism between the electrical disturbance and functional activity in the nerve.

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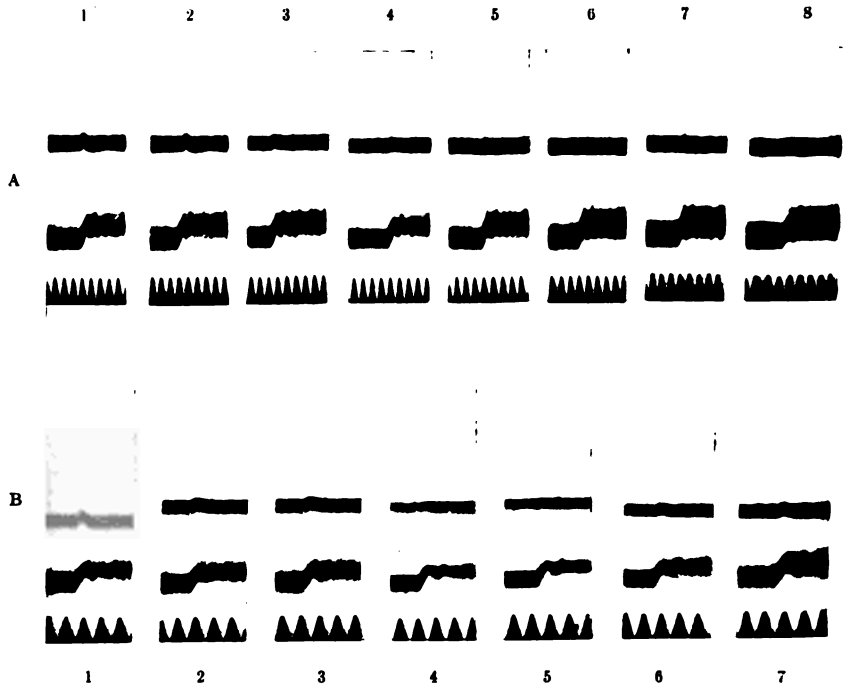


Fig. 5. Diphasic action currents of frog's sciatic nerve showing effect of ether vapor.

A. Experiment 4. *1*, before ether was introduced, contraction normal; *2*, two minutes after ether was introduced, contraction fair; *3*, three minutes of ether, contraction weaker; *4*, 3¾ minutes ether, contraction very weak; *5*, 4¼ minutes ether, no contraction; *6*, five minutes ether, no contraction. *7*, ½ minute after ether was removed from chamber, no contraction. *8*, 1½ minute after removal of ether, slight contraction.

B. Experiment 3. *1*, one minute of ether, normal contraction; *2*, 2½ minutes of ether, contraction weaker; *3*, three minutes ether, contraction very weak; *4*, 3¾ minutes ether, no contraction; *5*, four minutes ether, no contraction; *6*, 1½ minute after removal of ether, contraction small; *7*, two minutes after removal of ether, contraction small.

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Even if, as was intended, the action of ether was more intense near the stimulating electrodes than at the nerve ending, it is quite possible that the decrement imposed on the nerve impulse by the drug was such that it failed to pass the neuromuscular junction and excite the muscle, although persisting throughout the region where the galvanometer leads were applied.[16] On the other hand, the failure in any instance to obtain muscular contraction without a simultaneous action current in the nerve, although the ether was regularly introduced proximal to the leads, supports, so far as it goes, the view that the nerve impulse cannot occur without its concomitant electrical disturbance. At all events, it supports the findings in the case of general anaesthesia, and leads to the conclusion that it is impossible by any application of ether to abolish the electrical disturbance in nerve while the tissue remains functionally active. The action current is probably a valid criterion of function.

SUMMARY

1. As a preliminary to an investigation of the effect of ether anaesthesia on afferent impulses going to the brain, a study has been made of its effect upon the action current in a nerve trunk subjected to direct stimulation.

2. Two methods have been employed. One was to etherize a cat and see whether at any depth of general anaesthesia the action current in a motor nerve directly stimulated, could be abolished. The second was to apply ether vapor directly to an exposed frog's nerve and see whether at any time the action current could be abolished while the nerve was still shown to be functionally active by contraction in the innervated muscle.

3. We find that even when etherization in the cat is pushed to the point of abolishing respiration and causing death, the nerve trunk remains functionally active and exhibits what appear to be essentially normal action currents.

4. With direct application of ether vapor to the isolated nerve-muscle preparation, the action current in nerve regularly persists at least as long as contraction in the muscle, and in almost every instance longer.

5. The evidence, so far as it goes, supports the view that nerve impulse and electrical disturbance are inseparable. It leads us to believe that, at any rate, the action current is a safe criterion of the presence or absence of the nerve impulse in connection with the administration of ether.

[16] Adrian: Journ. Physiol., 1912, xlv, 389; 1913, xlvi, 385.

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A METHOD FOR MAINTAINING AN ARTIFICIAL CIRCULATION THROUGH THE TIBIA OF THE DOG, WITH A DEMONSTRATION OF THE VASOMOTOR CONTROL OF THE MARROW VESSELS

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INTRODUCTION

The inaccessible nature of the bone marrow seems to have preserved it from attempts at direct physiological study. With the exception of work by Franz Müller(1) we have found no efforts towards such an end. This observer in 1901 examined blood taken directly from the nutrient vein of the tibia, and found that if his operation was performed with no interruption of arterial flow and with no hemorrhage, this blood corresponded entirely with blood taken from other parts of the same animal. But if he clamped the nutrient artery from twenty to thirty minutes, the specimens then contained many normoblasts. The same result, though less marked, could be secured by making the animal breathe an oxygen poor atmosphere. The article aims to clear up certain phases of the problem of oxygen lack and red cell formation. It makes no mention of the other principal marrow product, the leucocyte. The full significance of these results will be discussed at a later date, since they seem to have a very direct bearing on the mechanical factors involved in the appearance of new cells in the circulation.

Müller also mentions the fact that nerves have been found passing to the marrow vessels. That the limbs possess a poor vasomotor mechanism as compared with the abdominal vascular area is common knowledge, but the possibility of a complete and very effective supply to the bone marrow has entirely escaped physiological observation. Gros (2), in 1845, first described the existence of such nerves. Variot and Remy (3), in 1880, attempted to amplify his observations. But the methods then at hand were not adequate for the task. Ottolenghi (4), in 1901, with the advantage of the methods of Golgi and Ehrlich made

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a very thorough study of the nerves to the marrow. He examined material from man, sheep, dog, guinea pig, rabbit and chicken, and concludes:—

1. The marrow is richly supplied with medullated and non-medullated fibres.

2. These nerves form fine plexuses in the walls of the blood vessels, many ramifications reaching the capillaries.

3. In the marrow pulp there are many medullated and non-medullated fibres passing eventually to distant vessels.

4. The existence of special nerve terminations about independent marrow elements cannot be settled.

Myelinated fibres in this region can only be afferent and suggest the possibility of specialized reflexes from the marrow.

METHOD OF PERFUSION

After many dissections the tibia of the dog was found to be the bone best suited for perfusion. Similar experiments can be done upon the femur but the operation is more difficult and the bulk of tissue perfused not so large. Dogs weighing about 8 kgm. are desirable but, if necessary, smaller animals may be used when the operator is thoroughly familiar with the anatomy of the part. A skin incision from a point 1 inch above the knee joint to a point 2 inches above the ankle joint, along the line of the inner border of the tibia, exposes the fascia above the bone and permits the operator to carry out his dissection along the inner margin. Care should be taken not to cut or tie the saphenous vein as it receives radicles from the lower extremity. The popliteus muscle is cut away from its insertion, and the popliteal artery and vein exposed as they pass down between the condyles of the femur. At all times the operator must keep close to the bone, cleaning off all the muscle attached to the periosteum, but not removing this membrane. The thick fascia covering the flexor communis digitorum is cut close to the bone and the origin of this muscle is carefully cleared away by blunt dissection. The nutrient artery usually leaves the popliteal artery as one branch of a short small trunk appearing about three-fourths of an inch below the knee joint, and runs obliquely downwards, passing under the tibial periosteum near the external border about one-third of the way down the shaft. It traverses the belly of the flexor communis digitorum, and when the origin of this muscle is removed the artery with the vein beside it will be seen. Occasionally

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the artery comes off independently somewhat lower down. The nerve to the marrow, a branch of the tibial, is extremely small and lies in close relation to the nutrient artery. Figure 1 indicates the relations which obtain after the dissection is completed and all the muscular branches in the neighborhood ligated. Having made such exposure, a cannula is placed in the popliteal artery below the egress of the nutrient artery, the perfusion started, and the fluid allowed to flow up the popliteal artery against the normal blood stream. A ligature which has been placed around the popliteal above the nutrient artery is now tied, and the perfusing fluid is shunted through the latter artery without at any time interrupting the flow of fluid to the bone. In order to eliminate the possibility that the reactions hereafter described are due to contractions of the nutrient artery outside the bone, a cannula of the type shown in figure 2 has also been used. With the perfusing fluid running, such a cannula is passed into the popliteal below the nutrient artery and its point gently guided up into the mouth of the nutrient artery, down which it is cautiously pushed until the point reaches the periosteum. There are no differences in the type of result obtained by these two preparations.

If such procedures are properly carried out is the marrow completely perfused?

The tibia in common with other long bones has three sources of blood supply:

1. By minute periosteal arteries from fascial and muscular twigs which pass near the bone.

2. By a number of moderate sized vessels which enter the extremities of the bone through small foramina usually in the line of the attachment of the joint capsules.

3. By the nutrient artery. This is unquestionally the main source.

At the present time there is no basis upon which to found a belief that perfusion through the nutrient artery will keep all the marrow alive, but we can ascertain that all the marrow, except at times the extreme upper posterior part of the tibial head, is thoroughly bathed by the perfusing fluid. The lower extremity receives practically no blood save by the nutrient artery. There are usually two and sometimes three very short small branches which pass directly from the popliteal artery to the upper posterior part of the head. These are hard to see in the living preparation as any disturbance of the popliteal artery breaks them. It is possible to take advantage of their presence by passing the ligature, which shunts the perfusing fluid into the bone.

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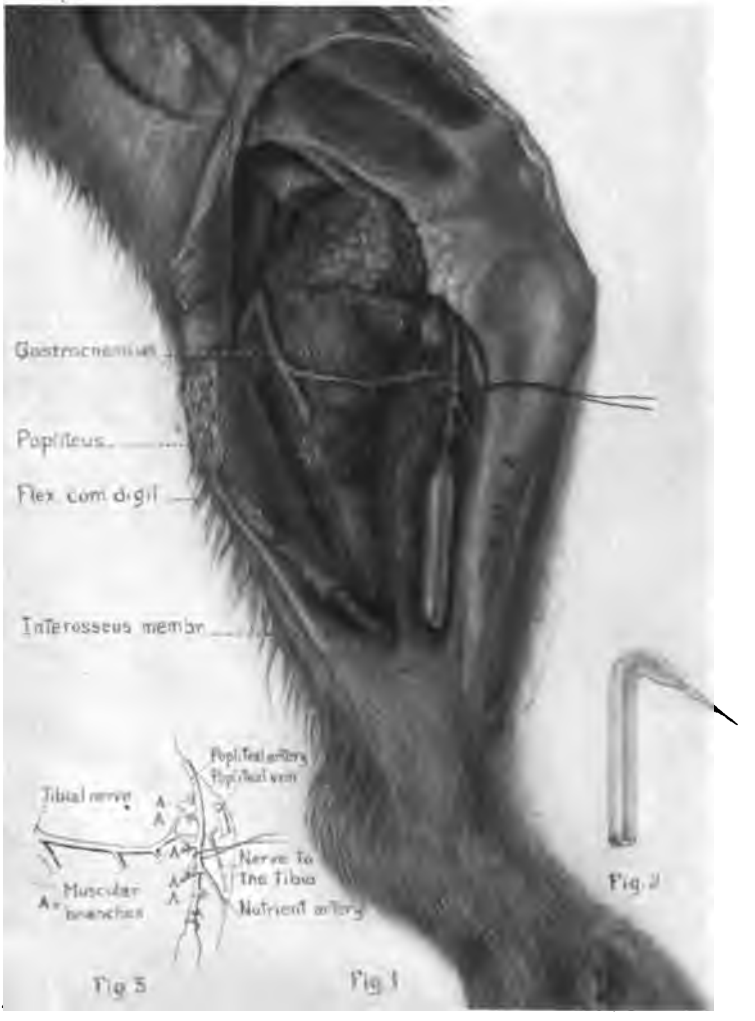


Fig. 1. Dissection of the nutrient artery and of the nerve to the marrow. The tibial nerve is displayed fully in order to show the origin of the nerve to the marrow.

Fig. 2. Special cannula for insertion into the nutrient artery.

Fig. 3. Key to figure 1.

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above the head of the tibia, but in the experiments here detailed this was not done.

The long bones possess three sets of veins corresponding to the arteries, and though the nutrient vein carries the main effluent, leakage from vessels throughout the periosteum is a constant difficulty. A preparation can be made in which the venous return can be kept intravascular throughout and can be collected from the vein, but this has been unnecessary in these experiments. In this work when the perfusion has been started the common iliac artery is tied, the tibia is disarticulated at the knee and ankle and is cut away from its muscular attachments. The isolated bone, to which a circulation has thus been uninterruptedly supplied, is then placed in a shallow trough with an outlet tube at one end. Now all inflow reaches the marrow through the nutrient artery and all outflow falls in the trough and drops from the outlet tube. We have made use of a simple form of constant pressure and continuous flow perfusion apparatus and have employed oxygenated Ringer's solution as the perfusing fluid. With such an apparatus variations in the outflow under constant inflow pressure can mean only variations in the calibre of the vessels perfused.

EXPERIMENTS

No. 19. Action of epinephrin. Dog, weight 14.2 kgm. Anesthesia; Luminal-Sodium* administered intraperitoneally. Perfusion with oxygenated Ringer's solution started at 3.51 p.m. Bone removed at once. Outflow recorded in drops.

Time	Drops of outflow in 30 seconds		Time	Drops of outflow in 30 seconds	
4.34	64	Pressure 115 mm. Hg.	4 46	6	
			4 48	11	
4 35	64		4 49	16	
4.37	64		4 52	47	
4.38	63		4 55	51	
4.39		Epinephrin 0.000,02 gm.†	4 57	52	
			4 58	53	
4 39[10]	11		5 00	52	
4 40[20]	4		5 01		Epinephrin 0.000,002 gm.
4 42	2				
4 43	2		5 01[30]	28	
4 44	1		5 02	4	

* Luminal-Sodium 5% gave entire satisfaction when given intraperitoneally dissolved in 0.9 per cent NaCl in the dose of 0.150 gm. per kgm.

† This and subsequent doses of epinephrin dissolved in Ringer's solution were injected through the rubber tubing leading to the inflow cannula. The dilution in which the drug reached the marrow vessels cannot be given.

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Time	Drops of outflow in 30 seconds		Time	Drops of outflow in 30 seconds	
5.03	2		5.49	41	
5.06	0		5.51		Epinephrin
5.20	5				0.000,000,2 gm.
5.30	44		5.51[30]	36	
5.44	40	Pressure 102 mm.	5.52	27	
		Hg.	5.53	28	
5.45	41		5.54	31	
5.48	42		5.58	44	

No dilatation with epinephrin could be observed under the conditions of this experiment. It must be recognized that the marrow vessels at the outset of such an experiment are in a condition of wide paralytic dilatation due to the severance of their nerve supply. This is probably exaggerated by constant pressure perfusion and by the poor oxygen carrying power of the Ringer's solution. A slight but rather doubtful degree of active dilatation was secured in another experiment in which ergotoxin phosphate was given to a dog until small doses of epinephrin gave a dilator reaction. At this point perfusion was started, the bone removed and epinephrin injected. The dilatation which resulted could be repeated by only one subsequent injection and could not be shown by any type of direct nerve stimulation. We have therefore no reliable evidence of an active dilator mechanism.

No. 26. Electrical stimulation of the nerve to the marrow. Dog, weight 13.6 kgm. Anesthesia; Luminal-Sodium administered intraperitoneally. Perfusion with oxygenated Ringer's solution.

Time	Cc. of outflow in 30 seconds		Time	Cc. of outflow in 30 seconds	
12.00	11.5	Pressure 125 mm.	12.05		Stimulation begun
		Hg.	12.05[30]	9.8	
12.01	11.0		12.06	3.2	Stimulation ended
12.02	11.0	Electrode in posi-	12.07[30]	5.0	
		tion	12.08[30]	10.0	
12.04	12.0		12.11	10.0	

In this case a very large flow was secured and the experiment is given to indicate the extent to which the flow can be reduced by faradic stimulation of the nerve.

No. 27. Electrical stimulation of the nerve to the marrow. Dog, weight 20.2 kgm. Anesthesia; Luminal-Sodium administered intraperitoneally. Bone removed in usual manner. Outflow record with drop recorder. Nerve stimulated with faradic current between marks *A* and *B*. Figure 4 gives the record obtained in this experiment and presents the ordinary characteristics of vasomotor reactions, i.e., slow onset and slow disappearance.

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It has been impossible to secure vasodilatation by slow weak stimulation. The mechanism is so sensitive that handling of the nerve, unless accomplished with the utmost precaution, causes constriction. The constrictor effect of epinephrin has been observed in eight different experiments and constriction on direct stimulation of the nerve has been observed in five. From these experiments we have given the above characteristic examples, and feel that they present ample verification of the existence of the large vasomotor supply which the anatomical investigations of Ottolenghi have indicated.

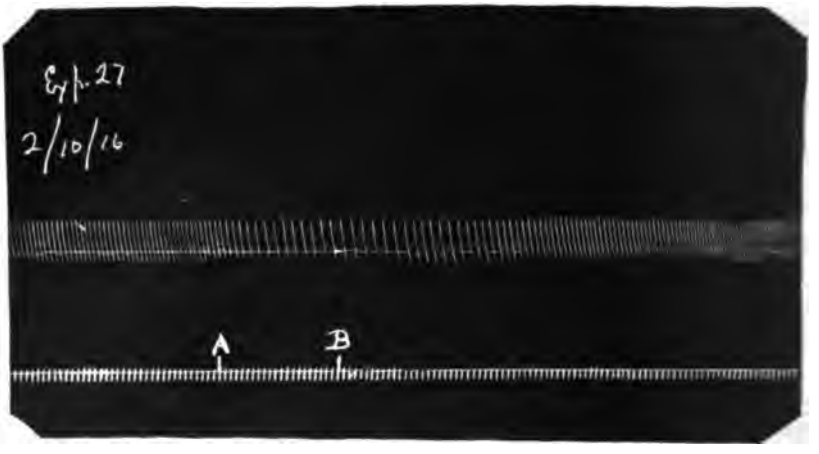


Fig. 4. Vasoconstriction on faradic stimulation of the nerve to the marrow. Stimulus applied between *A* and *B*. Upper line: drop recorder. Lower line: chronograph recording two second intervals.

Our present studies indicate that this intense nervous control has a marked influence on the cellular output of the marrow and experiments dealing with this aspect of the work are now in progress.

CONCLUSIONS

a. A method of perfusing the bone marrow is described.

b. The existence is demonstrated of vasomotor nerves to the marrow. These nerves respond on electrical stimulation and on injection of epinephrin by causing vasoconstriction.

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BLOOD PRESSURE IN HAEMORRHAGE AND ITS RESTORATION

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Haemorrhage lowers blood pressure, but the fall in pressure is not invariably proportionate to the amount of blood lost. As is stated by Pilcher and Sollmann, the relation of the fall of blood pressure to the amount of blood lost varies in each animal, but the median type is approached more or less closely in each case. As is further stated by the same authors, the low blood pressure level, or the blood pressure in shock, depends chiefly on the amount of blood lost and not to an important degree on the rapidity of the haemorrhage (1).

In performing the experiments that will be referred to in this paper we were actuated by a desire to learn just what results could be hoped for in endeavoring to restore the blood pressure to normal after haemorrhage. For experimental purposes rabbits were used. They were anaesthetized by the administration through the stomach tube of 1.5 cc. of paraldehyde per kilogram of body weight. The haemorrhage was accomplished by withdrawing from the femoral artery blood in the proportion of 5 cc. per kilogram of body weight, and continuing to withdraw equal amounts until the blood pressure was reduced to the desired level. Intravenous injections of normal saline solution were then made in amounts sometimes less than, in others equal to, and in still others greater than, the amount of blood lost.

In general it may be stated that removal from the circulation of 5 cc. of blood per kilogram is without influence on the blood pressure. Upon the withdrawal of the second portion of 5 cc. per kilogram blood pressure begins to fall and there is a fairly constant fall of pressure with the removal of each successive portion until 20 cc. or 25 cc. per kilogram have been withdrawn. The fall of blood pressure with the loss of each 5 cc. of blood per kilogram averages 6 mm. of mercury (Group 1). After 20 cc. or 25 cc. per kilogram have been removed the loss of more blood causes a more rapid fall in pressure. At this

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point we found that each 5 cc. of blood lost per kilogram caused an average fall in blood pressure of 10 mm. (Group 2), and when 35 cc. to 40 cc. per kilogram had been lost the animal was in a condition of shock with a blood pressure varying in different animals from 22 mm. to 35 mm. of mercury.

If normal saline solution be injected during the first stage of haemorrhage, that is while the blood pressure is falling slowly, there is a rapid and permanent return to normal. This may be seen by reference to the tabulated report of group 1.

Group I

EXPERIMENT	HAEMORRHAGE IN CUBIC CENTIMETERS PER KILOGRAM	5	10	15	20	25	NORMAL SALINE IN CUBIC CENTIMETERS PER KILOGRAM	BLOOD PRESSURE AFTER INJECTION
A1	Blood pressure, 80	80	77	72	66	60	30	75
A2	Blood pressure, 84	82	76	69	61	53	25	73
A3	Blood pressure, 81	80	73	64	55		15	76
A4	Blood pressure, 84	80	71	66	59		20	82
A5	Blood pressure, 82	81	75	69			10	79

The first blood pressure recorded is that at the beginning of the experiment, before any blood had been withdrawn. The figures given for blood pressure represent millimetres of mercury.

If the injection of normal saline solution be made during the second stage of haemorrhage, that is during the period of rapid fall in blood pressure, the permanent return of pressure to normal can be accomplished, but the response is much slower than in the first stage. Immediately following the saline infusion the pressure rises 20 mm. or even 30 mm. In one case it rose 38 mm. After the initial rise the return to normal is slow and requires from twenty-four hours to forty-eight hours.

Group II

EXPERIMENT	HAEMORRHAGE IN CUBIC CENTIMETERS PER KILOGRAM	5	10	15	20	25	30	35	SALINE IN CUBIC CENTIMETERS PER KILOGRAM	BLOOD PRESSURE
B1	Blood pressure, 81	80	73	65	58	50	39	28	45	58
B2	Blood pressure, 85	82	74	66	58	53	40	30	35	55
B3	Blood pressure, 80	80	73	67	60	52	46	35	30	73
B4	Blood pressure, 86	84	80	71	63	51	41		30	61
B5	Blood pressure, 82	79	68	59	47	39	30		40	48

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If the injection of saline solution be deferred until the third stage of haemorrhage, i.e., until collapse has occurred, the possibility of bringing about a permanent restoration of blood pressure is remote. The first effect of the saline injection is a rise of pressure, this rise usually being about 10 mm., though in one case that will be referred to again it was 20 mm. Repeated injections of large amounts of saline solution were without further effect under these conditions except in the case mentioned. In this case the method followed was the same as in four others. Blood pressure was reduced to 45 mm. of mercury by successive bleedings, the total amount of blood lost being 40 cc. per kilogram. Prompt administration was made of 50 cc. of saline solution per kilogram with rise in blood pressure of 20 mm. As the blood pressure began to decline a further injection of 50 cc. of saline solution per kilogram was given. A third injection of 30 cc. per kilogram was made with the result that there was a gradual return to normal. The course of this experiment is shown in group 3, experiment C4. The four other animals that suffered loss of the same amount of blood, except C2 which was bled to the extent of 35 cc. per kilogram, were treated in the same way and showed an initial rise of pressure of 10 mm. as an average. This soon began to decline and repeated injections of saline solution were without effect.

Group III

EXPERIMENT	HAEMORRHAGE IN CUBIC CENTIMETERS PER KILOGRAM	5	10	15	20	25	30	35	40	SALINE IN CUBIC CENTIMETERS PER KILOGRAM	BLOOD PRESSURE
C1	Blood pressure, 125	120	111	102	92	76	60	44	26	50	35
C2	Blood pressure, 128	124	113	100	85	74	55	35		50	41
C3	Blood pressure, 127	126	119	109	100	90	77	57	23	50	0
C4	Blood pressure, 120	119	111	99	90	80	71	59	45	50	65
C5	Blood pressure, 135	132	120	107	95	83	67	53	37	50	50

In group 3 cats were used instead of rabbits. They were anaesthetized by the administration of ether by inhalation. The initial blood pressure was higher than in the rabbits, but the results are comparable with those obtained in the first two groups of experiments. At first rabbits were tried but they succumbed very quickly after the removal of 35 cc. or 40 cc. of blood per kilogram. Then cats were resorted to with slightly better results as has been recorded.

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Pilcher and Sollmann explain the rise in blood pressure as due to stimulation of the vaso-motor centre causing vaso-constriction. They state that the vaso-motor centre is not affected by infusion of saline solution if the blood pressure be 60 mm. of mercury or above, but that when the blood pressure is below 60 mm. the vaso-motor centre may be stimulated by such injections (2). The author's results corroborate this assertion, at least so far as the influence of saline infusions when the blood pressure is below 60 mm. is concerned. No attempt was made to determine the effect of intravenous administration of saline solution at higher blood pressure levels as the experiments were undertaken for a different purpose. The object, as already stated, was to determine what could be hoped for in the way of restoring blood pressure and maintaining the circulation in cases of haemorrhage of varying degrees of severity. From these observations we draw the conclusion that so long as the haemorrhage has not been great enough to reduce blood pressure to the "shock level" gratifying results may be hoped for from the intravenous administration of normal salt solution. When the blood pressure has reached the level of shock, 30 mm. to 50 mm., restoration of blood pressure and maintenance of the vital functions of the organism are a possibility, but cannot be expected with any certainty. In general it can be stated that in haemorrhage injection of amounts of saline solution in excess of the amount of blood lost will give the best results; in severe cases the use of large amounts of normal salt solution, 50 cc. to 100 cc. per kilogram of body weight, is most likely to be attended by a successful outcome.

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STRUCTURE OF THE FIBRIN-GEL AND THEORIES OF GEL-FORMATION

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The usual view of the structure of the fibrin-gel is that it is composed of a fine reticulum of fibrin-threads, holding the water within its meshes. A reticulum of this character can be seen in fact with the microscope in a drop of coagulated blood as was first described by Ranvier, but recent observations demonstrate that this is not the primary mode of formation of the gel. This traditional fibrin net-work must be regarded as an artifact caused by mechanical tension or pressure exerted in the preparation. Stübel (1), making use of the dark field illumination discovered that in clotting the fibrin is deposited as separate nedles or crystals. The author (2), who was at the same time studying the process of clotting with the ultramicroscope, making use of the slit-form, was able promptly to confirm and extend Stübel's discovery.[1] The object of the present paper is to report further observations upon the structure and formation of the fibrin-gel.

THE CRYSTALLINE GEL

The mode of formation of the fibrin needles was observed in most cases by using the clear cell-free plasma obtained by centrifugaliz-ing oxalated mammalian blood. To specimens of this plasma thrombin, in saline solution, was added and the mixture was then introduced into the ultramicroscope cell for observation. The thrombin,

[1] Hekma (Internationales Zeitsch. f. physikalisch-chemische Biologie, 1915, 2, 354) calls attention to the fact that fibrin needles were observed many years ago by Schimmelbusch (Virchow's Archiv, 1885, 101, 201). He described them as very thin spindle-shaped needles, 5 to 20 micra long, which are formed independently of the corpuscular elements of the blood. His observations were overlooked apparently or were not confirmed by subsequent workers, but his figures show that he was dealing with the same structure whose existence is now revealed so easily and so unmistakably with the aid of the ultramicroscope.

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prepared by a method previously described (3), was kept in dried condition, a small portion being dissolved for use when needed. The mixture of oxalated plasma and thrombin clotted in a shorter or longer time according to the concentration of thrombin used, and it was easy to choose such a concentration as would induce clotting in a convenient time for observation. The oxalated plasma when possible was obtained from fasting animals and was therefore free from noticeable amounts of fat. Under the ultramicroscope it showed scattered large particles, probably fat granules, and a luminous cone, marking the beam of light, in which no visible particles could be distinguished. The colloidal material in the plasma exists, therefore, in a degree of dispersion not resolvable by the ultramicroscope, the particles coming under the general designation of amicrons. The thrombin solutions, in the concentration employed, showed under the ultramicroscope a number of scattered large particles which presumably, on account of their small number, are to be considered as foreign material. For the rest the field was almost optically empty with a faint indication of the presence of a luminous cone.

The appearances observed, when the two solutions were mixed in proportions sufficient to cause clotting, varied according to conditions. If the amount of effective thrombin was for any reason insufficient to cause prompt or complete clotting one might observe first a certain increase in the luminosity of the opalescent cone, and later separate minute needles of fibrin appeared here and there in the field, coming into view quietly and floating slowly in one direction or another. If the clotting was imperfect these needles never became numerous enough to cohere into a solid mass. Under such circumstances the mixture when removed from the observation cell would show no visible clot. If, however, the amount of thrombin was sufficient to give a perceptible clot the formation of needles, once it had begun, would proceed steadily until they formed a meshwork of intermingled crystals. In mixtures that clotted firmly in from five to ten minutes the formation of the crystals began somewhat suddenly over the whole field and proceeded rapidly until the field was converted into a mass of the intermeshed needles. The intermediate steps in the formation of the needles were followed most successfully when the plasmas were much diluted with salt solution (sodium chloride 0.9 per cent) or when fibrinogen solutions were employed in place of the oxalated plasma. Under these conditions the steps that could be made out were as follows. First, an intensification of the luminosity of the opalescent cone, which we may

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assume was due to a beginning aggregation of the amicrons into particles of larger size. Second, a granulation of the whole field. Brilliant shimmering particles appeared throughout the whole cone, and these particles exhibited not only the usual Brownian movements shown by particles of such size, but in many cases also abrupt almost jumping movements which took them into or out of focus and gave to the whole field the appearance of an agitated scintillating mass. The particles rapidly assumed the visible shape of small rods, like short bacilli, and then grew into the longer acicular crystals. With the increase in size the abrupt and the Brownian movements were diminished and the final picture was the mass of brilliant stationary needles closely intermeshed. The inference to be made from these appearances is that the fibrin needles are formed by the aggregation of the amicrons of the fibrinogen solutions, and that in this aggregation or precipitation under the influence of the thrombin a vectorial force is brought into play which controls the agglutination of the particles into definite crystal-like needles. Fibrinogen may be precipitated out of its solutions in various ways, by the action of heat, of neutral salts, dilute acids, etc., but in all such cases the fibrinogen particles are aggregated into amorphous clumps, whereas under the influence of thrombin the particles are arranged in accordance with a directive force which is developed in the interaction of the thrombin and the fibrinogen.

The deposition of fibrin needles when oxalated plasma is clotted with thrombin has been observed with hundreds of specimens of blood from human beings under normal and pathological conditions and from various mammals. An objection may be made possibly to the use of the isolated thrombin in these cases and some doubt may be raised as to whether or not the thrombin as it normally occurs in the blood has a similar action.

To meet this possible objection the following methods were employed to insure the clotting of the plasma by means of its own thrombin.

1. The blood as it flowed from the artery was received into a 20 per cent solution of sodium or potassium chloride in proportions to yield a mixture contain 5 per cent of the salt used. This concentration of salt prevents coagulation. The mixture may be centrifugalized to obtain a cell-free plasma and this plasma clots readily on simple dilution with water. Specimens of this kind observed under the ultramicroscope showed a typical deposition of fibrin-needles.

2. Oxalated and centrifugalized plasmas clot readily and firmly on

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the addition of a suitable amount of calcium chloride. If the amount of calcium chloride is properly chosen the resulting precipitate of calcium oxalate may be centrifugalized off and the clear supernatant plasma may be introduced into an observation cell before clotting occurs. When the clot forms under these conditions it is accompanied by a dense deposition of fine needles.

3. Slow clotting bloods, such as that of the bird, may be centrifugalized to remove corpuscles, and the clear plasma which clots spontaneously after a long time, may be introduced into the cell for observation under the ultramicroscope. Here also the clot when it forms is found to consist of a meshwork of fibrin needles.

There can be no doubt therefore that the normal process of clotting, in the mammalian blood at least, results in the formation of needle-like crystals of fibrin, which are formed separately but which later become intermeshed and possibly fused together. The normal blood-clot is a crystalline gel. It is a matter of interest to inquire whether the clot shows this structure in the blood of all animals. I have examined the blood of several mammalia (man, dog, cat, rabbit, horse, pig), of the bird (hen), of the reptile (terrapin) and of the invertebrate, the crab.

Among the mammals it can be shown easily, by the methods given above, that normal clotting consists in a deposition of fibrin needles, but undoubtedly the process is more easily disturbed or altered in some bloods than in others. For example, when the plasma (oxalated and centrifugalized) of the cat's blood is diluted four times or more with saline (0.9 per cent sodium chloride) and then clotted with thrombin a structureless clot may be obtained in which no fibrin needles occur, particularly if the amount of thrombin is large enough to cause prompt clotting. With dog's blood or human blood on the contrary dilution even a hundredfold with saline causes no such effect; up to a high degree of dilution addition of thrombin causes the formation of needles, indeed the details of the process of formation of these needles can be seen more clearly in some respects when the plasma is highly diluted. In the blood of the bird and the terrapin some difficulty is found in inducing coagulation conveniently in the oxalated and cell-free plasmas. As is well known the bloods of these animals clot very slowly, when protected from any admixture with tissue-juice, owing, as the author believes, to the relatively large excess of antithrombin present. Owing to this excess of antithrombin the centrifugalized oxalated plasma of such bloods does not clot readily upon the addition of thrombin solutions, and the animal's own blood serum is even less satisfactory for

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this purpose, since, unless perfectly fresh, it may contain no effective thrombin. The thrombin in the sera of these animals passes into the ineffective metathrombin stage much more quickly than in the case of mammalian blood. In a number of ways however it may be demonstrated that the reptilian and the avian blood like the mammalian blood gives fibrin crystals on clotting. The most conclusive proof that this constitutes the normal clot in these bloods is obtained by allowing them to clot spontaneously in the ultramicroscope chamber. By using paraffined canulas and receptacles the blood of the hen or terrapin can be drawn off and centrifugalized without clotting. The normal cell-free plasma may then be pipetted off, placed in the observation cell and allowed to clot spontaneously. Under such circumstances the clot shows a typical crystalline structure as in the case of mammalian blood. When oxalated bird's plasma was used, after centrifugalization, and the thrombin was added in the usual manner no clotting en masse was obtained owing to the large amount of antithrombin present. But although in such a specimen no visible gel was formed, nevertheless, in the ultramicroscope chamber it could be observed that separate fibrin needles were deposited. In accordance with the slowness with which the process occurred these needles were unusually long, but they were scanty in number and entirely separate floating slowly into and out of the field of vision. Fibrinogen prepared from the oxalated plasma of the bird or terrapin by the usual method of successive precipitations by half-saturation with sodium chloride gave a typical crystalline gel upon the addition of thrombin, provided at least two precipitations were made of the fibrinogen with the usual precautions of washing the precipitate. The fibrinogen obtained by a single precipitation of the plasma might fail to give a clot with thrombin, owing no doubt to the excess of antithrombin present in the original plasma and still contained in the first precipitate of fibrinogen. These results it may be noted give a striking proof of the non-specificity of thrombin. The thrombin used was prepared in all cases from the fibrin of pig's blood and it was effective in causing a typical crystalline gel with the plasma or the fibrinogen from any other mammalian blood or apparently with the blood of any vertebrate animal. It should be stated however that in the plasma of both the bird and the terrapin the fibrinogen can be altered or denatured more readily than in the case of the mammalian blood. Simple dilution of the plasma or, in the case of the bird, the presence of much fat in the blood seemed to alter the fibrinogen so that the gel produced by thrombin differed in its

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physical properties and ultramicroscopic structure. Fibrinogen is an unstable protein which is readily denatured to a greater or less extent by variations in reaction and changes of other kinds, and it would seem that in this susceptibility to alteration in properties the fibrinogen of the bird and reptilian blood is more unstable than the similar protein occurring in mammalian blood.

While all of the vertebrate bloods examined give a crystalline gel when the conditions of clotting are normal this was not found to be the case in the blood of the crab. The blood of this animal gives a firm clot when removed from the body. If a specimen of the blood is taken from the heart by means of a syringe and transferred to the observation cell normal clotting occurs and the structure of the clot may be examined ultramicroscopically. Specimens obtained in this way showed no structure whatever. There were a few scattered large particles, but the blood before and after setting to a gel showed simply a luminous cone in which no visible particles could be made out. The gel was wholly structureless so far as the ultramicroscope could determine. Since the crab's blood gives an alkaline reaction it was thought, in accordance with the facts described in the next paragraph, that this might explain the lack of structure in the clot, and that in a neutral or slightly acid reaction fibrin needles similar to those of mammalian blood might be obtained. In one experiment therefore decinormal hydrochloric acid was added to the blood taken from the heart in the proportion of three drops of the acid to 1 cc. of the blood. The acid causes an abundant precipitate in which the corpuscles are entangled. By filtering a clear liquid is obtained which takes on a blue color and soon sets to a stiff non-retractile clot. Specimens of this plasma allowed to coagulate in the observation cell gave a clot which exhibited a fine granular structure, the particles showing some tendency to form short beaded threads. There was however no indication of the formation of fibrin needles such as are given by the vertebrate blood. That there is an essential difference in the properties of the fibrinogens of vertebrate and invertebrate (crab) blood is shown also by their reactions with thrombin. The blood of the vertebrates, so far as I have examined them, show little or no indication of a specificity in regard to thrombin. The oxalated plasma or the isolated fibrinogen of any of these bloods is readily clotted by the pig's thrombin, although in the case of the plasmas of the bird and the reptile it may be necessary to neutralize the excess of antithrombin by the addition of thromboplastic material (kephalin solution). Crab's blood or fibrinogen prepared from it by

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the usual method is, on the contrary, wholly unaffected by the mammalian (pig) thrombin used in these experiments. The process of blood coagulation among the vertebrates is essentially the same throughout, although minor differences in the properties or reactions of the fibrin-factors may be demonstrated in the different members, but among the invertebrates, if we may generalize from the results with crab's blood, the fibrin factors are of a different character and the gel formed by their interaction has a different structure.

THE STRUCTURELESS GEL

Under certain conditions mammalian fibrinogen may be made to give a structureless gel with thrombin. The gel in these cases is characterized by an entire lack of structure under the ultramicroscope, by its transparent appearance, by a diminution in or entire lack of retractility and by its easier solubility in dilute acids. I have been able to obtain these structureless gels by several different methods which may be described briefly.

1. *By the action of alkalies.* Fibrinogen solutions that give a typical crystalline gel with thrombin may be made to give a structureless gel if treated with a dilute solution of sodium carbonate or sodium bicarbonate in amounts sufficient to give an alkaline reaction on the addition of phenolphthalein. If the amount of alkali added is too large or it is allowed to act for too long a time the fibrinogen may be so altered that it will fail to clot at all with thrombin, but with the proper degree of alkalinity the formation of a clear structureless, non-retractile clot may be obtained readily. The same alteration in the fibrinogen may be produced by adding sodium carbonate (Na_2CO_3 0.25 per cent) to oxalated blood plasma. In this case much more of the carbonate must be added to obtain a distinct alkaline reaction with the phenolphthalein. In both cases, whether the oxalated plasma or the fibrinogen solution is used, the fibrinogen may be restored to its original condition in which it yields a crystalline gel with thrombin by simply precipitating it from its solutions with dilute acid or strong solutions of sodium chloride and redissolving the precipitate in a dilute (1 per cent) solution of sodium chloride, in the latter case, or by the addition of a drop or two of weak alkali ($HNaCO_3$, 0.5 per cent) in the former case.

2. *By standing.* If the oxalated and centrifugalized plasma is allowed to stand, in a refrigerator, for several days the fibrinogen is altered so that on addition of thrombin a structureless gel is formed.

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The time necessary for this change to occur varies with individual bloods, usually it requires from five to seven days, but it may take place, with cat's blood especially, in as short a time as seventy-two hours. As in the case of the alkali treatment prolonged standing results in a more complete denaturing of the fibrinogen so that it fails to clot at all with thrombin. In the intermediate stage in which it gives a structureless clot the fibrinogen of the plasma may be restored to its original normal condition by precipitation with neutral salts or weak acid and appropriate resolution.

3. *By dilution.* As mentioned above cat's plasma if diluted about fourfold with saline (sodium chloride 0.9 per cent) undergoes this alteration, especially if the proportion of thrombin used is large. Human and dog's plasmas are not affected in the same way by dilution. The fibrinogen precipitated from the cat's plasma is not altered in its properties by simple dilution.

4. *By drying.* The author has made frequent use of dried plasmas in his experiments. They are prepared by dialyzing the oxalated and centrifugalized plasma against a large volume of saline (sodium chloride 0.9 per cent) to get rid of the excess of oxalate, and then drying down the dialyzed plasma in small lots in watch glasses. The dried specimens are kept in a desiccator. They undergo a gradual deterioration with time. When freshly prepared they dissolve readily in saline solution and give a firm clot on the addition of thrombin. In fresh specimens the clot may show fibrin needles more or less perfectly formed. Later the clots pass into the structureless variety and in specimens that have been kept in the desiccator for many months the fibrinogen becomes so altered that it gives only an imperfect clot or none at all when thrombin is added. With the freshly prepared material it was often observed that solutions made with distilled water gave a crystalline gel with thrombin while those made with a solution of sodium chloride gave a structureless gel. The excess of sodium chloride seemed to act as a weak alkali. In this last case if the solution in sodium chloride was precipitated with weak acid and redissolved by the addition of weak alkali it would show fibrin needles when thrombin was added.

5. *By the administration of emetin chloride or of oxidized epinephrin.* In the course of experiments made by Drs. Levy and Rowntree (4) upon the effect of intravenous injections of emetin chloride it was noticed in some cases that the blood taken just before death not only showed delayed and imperfect coagulation but gave also a non-retractile

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clot. Some of this blood was therefore oxalated and centrifugalized and the clear plasma was clotted with thrombin in the observation cell. In some cases fibrin needles more or less imperfect were obtained, while in other cases the clot showed no structure and these last cases were the ones in which the clot also exhibited loss of retractility.

Somewhat similar results were obtained in a series of experiments made with Mr. Sosman upon the effects of intravascular injections of oxidized epinephrin. While lethal doses of epinephrin had no uniform effect upon the coagulation of the blood, injections of a certain oxidation product caused a marked delay in the time of coagulation and in some cases so changed the fibrinogen that a structureless instead of a crystalline clot was obtained by adding thrombin to the oxalated plasma.

In both of these cases of intoxication the blood, as tested with neutral red, gave indications of the development of a condition of acidosis, but this condition could not have caused directly the change in fibrinogen. Acidosis produced in other ways was not attended by any alteration in the structure of the clot.

These observations were of a more or less incidental character and were not extended by experiments with other organic bases, but they suggested the possibility that in some pathological conditions in man a similar change in the character of the clot might occur. With this idea in mind observations were made upon the blood of a great variety of patients in the Johns Hopkins Hospital. The blood was collected by venepuncture, oxalated and centrifugalized, and the clear plasma was then clotted in the observation cell by the addition of a solution of thrombin. The results need not be described in detail as they were wholly negative. In all the pathological bloods examined so far, including cases of pernicious anemia, cardiac and renal cases, typhoids, pneumonias, hemophilics, purpurics, secondary anemias, etc., the blood plasma on clotting gave a typical crystalline gel.

Of the several methods enumerated above by means of which the fibrinogen was so altered as to give a structureless gel with thrombin the simplest is the first in which the hydroxyl-ion concentration was increased. It is possible that in the other methods the same end result was obtained. An increase in hydrogen-ion concentration leads to an increasing aggregation and finally a precipitation of the colloidal particles of fibrinogen. Thrombin added at any time short of the actual precipitation gives fibrin needles and a crystalline gel. An increase in hydroxyl-ion concentration on the contrary causes a

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greater degree of dispersion and stability in the solution of fibrinogen, the gel formation with thrombin requires a longer and longer time for its completion and finally fails entirely. At some point in this increasing alkalinity, approximately at a hydrogen-ion concentration of $H = 10^{-9}$ the fibrin needles fail to appear and the gel becomes structureless. In intermediate stages the needles lose their sharp outlines, take on the appearance of broken beaded filaments or short rows of gran ules, while the gel that is formed loses correspondingly its property of prompt retraction.

It is a matter of interest in connection with the problem of gel-formation to call attention to the fact that when alkali is added to a fibrinogen solution or a blood-plasma in amounts sufficient to prevent the thrombin from causing any visible clotting there may still be an effect in the direction of a marked increase in viscosity. That is to say the thrombin exerts an influence upon the fibrinogen which causes the latter to unite with or bind the water to some extent, as shown by the increase in viscosity, although for some reason, possibly an increased degree of dispersion, this action does not go far enough to form an actual gel. The following method was used to establish this fact.

Specimens were used of oxalated and centrifugalized plasma of the dog and of fibrinogen prepared from this plasma. Sodium carbonate was added to the fibrinogen solution and plasma in amounts sufficient to prevent the subsequent addition of thrombin from giving a visible clot of any kind. The viscosity of these solutions, with and without the addition of thrombin, was determined by the use of Hirsch and Beck viscosimeter tubes at a constant temperature and under the action of gravity. In all cases the addition of the thrombin caused a distinct increase in viscosity. As an example the following case may be cited.

Mixture A. Oxalated plasma, 2.5 cc.; sodium carbonate, 5 per cent, 0.5 cc.; thrombin solution, heated for 5 minutes at 80°C. to destroy its efficacy, 1 cc.

Mixture B. Oxalated plasma, 2.5 cc.; sodium carbonate, 5 per cent. 0.5 cc.; thrombin solution, 1 cc.

These mixtures were allowed to stand for 1 hour, 15 minutes, and the flow through the viscosimeter tubes was then measured at a temperature of 17.5°C. Time of flow for A = 69 seconds. Time of flow for B = 79 seconds.

The specimens were allowed to stand for an additional four hours and determinations were again made, the temperature of the bath being

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19°C. Time of flow for A = 65 seconds; Time of flow for B = 77 seconds.

The retraction of the fibrin-gel. The retractility or contraction of the blood-clot is one of its best known and most important properties. Various observers have attempted to connect this property with the presence of blood-plates. Le Sourd and Pagniez (5) state that in the blood of an animal made plateless by immunization the clot loses its power of retraction. But that the plates have no necessary causal connection, mechanical or otherwise, with the retractility of the clot seems to be shown clearly by the fact that cell-free oxalated plasmas or solutions of pure fibrinogen made to clot by the addition of thrombin show this property to a marked degree. Its existence may be obscured by the adhesion of the clot to the walls of the containing vessel, but if the clot is loosened from the walls it quickly contracts forcing out a clear serum. In normal blood the plates may be connected indirectly with the phenomenon of contraction in that they serve as a source of thrombin and thromboplastic substance, and the firmness and retractility of the clot are increased with the concentration of these factors. The observations described above in regard to the differences in properties between the crystalline and the structureless gel seem to show that the phenomenon of contraction, the syneresis of the clot, is connected directly with the existence of the fibrin-needles. In the wholly structureless clot the gel is soft and transparent. It divides easily into pieces or fragments which may again flow together, but there is no indication of retraction or the formation of an expressed serum. The crystalline gel on the contrary shows always a marked tendency to contract. Even in very dilute solutions in which the fibrin is deposited as a delicate membrane, contraction is shown distinctly when the membrane is detached from the walls or is shaken gently; it shrivels up promptly to a much smaller membrane-like structure. It seems most probable that the contraction of the normal clot is an instance of the gradual change or aging of the colloided aggregate and is referable to a process of further condensation in the particles composing the needles. Contraction is a phenomenon exhibited by many gels, but certainly it is much more marked in the blood clot, especially of the mammal, than in the gel of gelatine, agar-agar or casein, or in the structureless gel of the crab's blood. The development in the vertebrates of a fibrinogen capable of yielding a crystalline retractile gel and the increasing perfection of this property in the higher vertebrates are explicable possibly in terms of a more perfect adaptation of this form of clotting to the prevention of hemorrhage.

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CATAPHORESIS EXPERIMENTS

The precipitation of fibrinogen by thrombin suggests a reaction between oppositely charged colloids and in accordance with this suggestion a number of experiments were carried out to determine the electrical charges if any, carried by these substances. The device used was essentially that described by Michaelis (6). Connecting tubes were employed containing agar-agar made up in one per cent solution of sodium chloride as used by Field and Teague (7). See Figure 1.

A current of 110 volts was used with a current transmission varying from 1 to 10 milliamperes according as the outside tubes, 1 and 2, contained water or a solution of sodium chloride (0.9 per cent). The non-polarizable electrode on the positive side was made of silver immersed in a solution of sodium chloride to prevent the passage of the silver ions into the solution in tube 1. On the negative side the electrode combination was zinc and zinc sulphate.

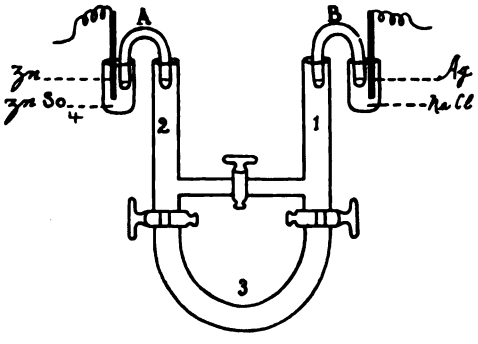


FIG. 1. Apparatus for Cataphoresis. Tubes 1 and 2 filled with water or with sodium chloride 0.9 per cent. 3 filled with the oxalated plasma. *A* and *B* connecting tubes filled with agar-agar made up in solution of sodium chloride 0.9 per cent.

So far as the thrombin is concerned it was found that in aqueous solutions of my purified thrombin, containing a little sodium chloride, some of the thrombin carried a positive charge. That is to say, after a cataphoresis lasting for an hour or more, with water in tubes 1 and 2, thrombin could be detected in tube 2 but was absent from tube 1. In serum, on the contrary, and in oxalated plasmas (prothrombin) that is, in slightly alkaline media, the thrombin and prothrombin exhibited a negative charge. They could be detected in tube 1 but not in tube 2. Since in such media the fibrinogen also exhibits a negative charge it would appear that the precipitation of the fibrinogen by the thrombin can not be referred to a reaction between oppositely charged particles.

Experiments of a similar character made upon oxalated blood-plasma after centrifugalizing off the corpuscles have yielded some sug-

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gestive results. In these experiments the outside tubes 1 and 2 were filled with 0.9 per cent solution of sodium chloride and the current transmission varied from 4 to 5 milliamperes. With this arrangement it was frequently possible to effect a separation of the fibrinogen in the plasma, some of it going to the negative and some to the positive pole, indicating therefore the existence in the plasma of some positively charged and some negatively charged fibrinogen. This separation was obtained most certainly when the reaction of the plasma was brought to or toward the neutral point and the current was passed for a short time, about one hour. A drop of neutral red solution (0.25 per cent) was added to 10 cc. of the plasma and then $\frac{N}{10}$ HCl until the orange yellow color took on a distinct reddish tint. The two kinds of fibrinogen obtained from tubes 1 and 2 reacted quite differently to thrombin. The positively charged material that had accumulated in tube 2 gave with thrombin a flocculent precipitate which in some cases settled to the bottom, but in other cases adhered to form a membranous mass resembling a so-called membranous clot. In no case was there a formation of a gelatinous clot. Examined under the ultra-microscope the material in tube 2 showed very numerous coarse particles. Under the influence of the thrombin these particles agglutinated to form clumps or short strings in which the separate particles were distinctly visible. The thrombin in this case acted after the manner of an agglutinin. The negatively charged material that was carried over into tube 1 gave always with thrombin a gelatinous clot. Examined under the ultra-microscope this clot exhibited in some cases the presence of typical thrombin needles, but in other cases, especially with those specimens of plasma which had been neutralized with dilute acid, the clot showed only a few scattered short needles or rods. For the most part it was structureless or showed only faint indications of nebulous masses in which no particulate structure could be made out. Its characteristics in fact tended to approach those described above as the result of the initial action of alkalies on fibrinogen. The plasma remaining in tube 3 at the end of the experiment gave always with thrombin a typical crystalline gel. These results suggest that the fibrinogen particles or aggregates may adsorb both hydrogen and hydroxyl ions. Adsorption of hydrogen ions tends to cause the precipitation of the fibrinogen particles by increased aggregation. The aggregates thus formed are still further influenced by thrombin to agglutinate into larger masses, but they do not exhibit the property of gelatinization. The adsorption of hydroxyl ions tends to the formation of a structureless gel. The dif-

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ference in reaction to thrombin exhibited by these oppositely charged fibrinogens suggests moreover an explanation of a peculiarity in clotting which has attracted the attention of many observers. In the clotting of solutions of fibrinogen with purified thrombin or in the clotting of specimens of plasma containing but little effective thrombin, in consequence for example of the presence of excess of antithrombin, it is noticed often that the clotting takes place in two stages. There is formed first a delicate membrane which on shaking quickly retracts to a small clump. Later a gelatinous clot forms. According to conditions the interval of time between the two stages may be brief or may amount to an hour or more. In terms of the suggestion made above it may be supposed that the first stage represents an action of thrombin on the fibrinogen particles that by adsorption of the hydrogen ions carry a positive charge, while the later gelatinization represents the slower reaction of the thrombin with the fibrinogen combined with the hydroxyl ions, in accordance with the general fact that the time of clotting is prolonged by an increase in alkalinity.

DISCUSSION

The formation of the gel. The peculiar gel formed by fibrin is an outspoken heterogenous system. The more solid phase, the fibrin-needles, is clearly separated from the liquid phase. But there is no indication at all of a merely mechanical inclusion of the water or external phase between more solid walls or septa such as is assumed in the net-work theory and especially the honeycomb theory of Bütschli. Hardy (8) has reported such a structure in gels of egg albumin, gelatine and India rubber, visible under the microscope as a solid framework holding liquid in the interstices. In a ternary system composed of water, gelatine and alcohol, or water, gelatine and corrosive sublimate he obtained a net-structure or honeycomb structure according to the concentrations used, and in a binary system of water and gelatine, 1.5 per cent, a similar honeycomb structure was observed when the solution was cooled to − 1°C. This view seems to have prevailed generally. Freundlich for example in his "Kapillar Chemie" defines gels as diphasic systems consisting of very thin connecting walls of solid amorphous substance, enclosing spaces filled with liquid. But recent observers who have made use of the ultramicroscope have obtained no evidence of any structure of this kind. Bachmann (9), and Zsigmondy and Bachmann report their observations upon the

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ultramicroscope study of the gels of silicic acid, agar-agar and gelatine. In dilute solutions of gelatine (1 to 6 per cent), while in a liquid condition, no definite structure can be seen. Outside certain optical impurities the field shows simply a cone of light due to the invisible amicrons. As gelatinization occurs there is a glimmering or sparkling movement in the cone resulting finally in the production of a swarm of minute submicrons. These submicrons are formed presumably by the aggregation or massing of the amicrons. When first formed they show translatory movements, but later only movements of oscillation that become less and less extensive until finally the particles are bound together in gelatine flocks which are stationary. The process is essentially the same as in the formation of the fibrin-gel described above, except for the appearance in the latter of the force which leads to the combination of the particles to form definite needles instead of amorphous flocculi. The important fact that remains to be explained is the binding of the water which leads to the solidification or gelatinization of the solution. As stated above the view that the water or more liquid phase is included between the walls of a solid phase, as in a sponge or honeycomb, finds no support at all in the results obtained from ultramicroscopic examination. Pauli (10) in an interesting paper has shown that in solutions of the emulsion colloids the viscosity increases with the ionization of the molecules. He assumes that in protein solutions the albumen ion undergoes hydration to a greater extent than the isoelectric molecules, hence the increased viscosity, and he suggests further that in gels we have simply an extension of this phenomenon, the solvent or disperse-medium being bound in great part as a hydrate. Obviously this theory of the binding of the water by the molecules or ions is not applicable to a heterogeneous system like the fibrin-gel. The fibrin-needles like other protein crystals possess probably the property of taking up water. Katz (11) has shown recently that in such cases the water is absorbed into the crystals in solid solution and not as a hydrate or as water of crystallization. But this process does not in any way explain the binding of the water surrounding the crystals. Other forms of crystalline gels are known. Zsigmondy and Bachmann (l. c.) have described such gels in the case of the soaps of the alkalies with the saturated or unsaturated fatty acids. As these solutions are cooled they form gels and the soaps separate out as needle crystals or fibers. Strong solutions of caffein are said to give a similar crystalline gel on cooling (Mathews). But the most complete analogy to the fibrin-gel is found in the interesting observations

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published by Flade (12). If equivalent amounts of barium hydroxide and malonic acid are dissolved in methyl alcohol the addition of glycerine to the system causes the formation of a gel, slowly or quickly according to the concentrations used. The gel-formation is accompanied by the deposition of needle-crystals of barium malonate visible under the microscope but seen especially well with the ultramicroscope These crystals as pictured resemble very closely in form and arrangement the mesh of fibrin-needles obtained in the clotting of blood. The crystalline gel of barium malonate like that given by the alkaline soaps differs in one respect from the crystalline fibrin-gel. In the former we may assume that the crystals separate out from a saturated solution, and that in the liquid phase the solute is present in strong concentration. In the formation of a fibrin-gel on the contrary the fibrinogen will separate out completely from highly-dilute solutions. If one starts with pure solutions of fibrinogen none of the solute remains in solution in the liquid phase. In this case, therefore, it would seem to be necessary to conclude that the binding of the water can not be connected with any dispersed phase other than the fibrin-needles themselves. The surface energy at the interfaces between the crystals and the water-phase must be responsible for the solidification of the gel. Some authors who have taken this view refer the matter to the surface tension of the medium, which gives to the small water-films the properties of a solid. But it seems very doubtful whether this view offers an adequate explanation. Metallic sols of any degree of dispersion fail to exhibit the property of gelatinization. Very little seems to be known of the conditions of surface tension between the solid and liquid phases in such suspensoids. It is known that the surface tension at the water-air contact is not affected by the suspensoids, and it is to be presumed that in the hydrophobic colloids like the metallic sols the surface tension of the liquid phase at its contacts with the solid phase is greater than in the case of the hydrophilic colloids in which the separation of the two phases is less distinct. The property of gelatinization is however a characteristic of the hydrophilic and not of the hydrophobic colloids, and since in the latter the surface tension in the water films is probably greater than in the former this consideration would seem to exclude surface-tension of the water phase as the underlying cause of gelatinization. In accordance with this reasoning we are forced to seek for the cause of the solidity of the gel in the surface action of the solid phase. The fibrin needles bind the water by virtue of the molecular attraction or adhesion between their

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surfaces and the water-molecules. This is presumably what Flade means when he states that the water in his gel of barium malonate is held by capillarity in the meshwork of crystals. From what has been said above in regard to the structureless fibrin-gel as compared with the crystalline gel it is evident that the gelatinization of the fibrin does not depend upon the existence of fibrin crystals. It may be assumed however that it does depend upon the existence of fibrin-aggregates. Under the influence of thrombin aggregates of this character are formed. Under certain conditions, neutral or weakly alkaline reaction, the aggregates unite to form the fibrin needles. In liquids of a stronger alkalinity the needles are not formed, but a gel is produced of a greater or less degree of firmness according to the hydroxyl concentration. The cause of the solidification of the blood-clot is to be sought in the special molecular attraction between the fibrin-aggregates and the water, and there seems to be no reason why this view should not apply to other gels such as those of silicic acid, gelatine and agar. It is in fact the view that was advocated years ago by Nägeli (13). "Die Micelle sich in Ketten an einander anhängen und ein Gerüste von Balken mit weiten Maschen bilden in welchem das wasser eingeschlossen ist und durch Molekularanziehung zwar nicht in einem ganz unbeweglichen aber doch in einem weniger beweglichen Zustande festgehalten wird." In the newer terminology we can substitute amicrons for "Micelle," and ultramicroscopic studies show that "Gerüste von Balken" are not a necesary structure of gels. The amicrons of the hydrophilic colloids may be massed or aggregated in amorphous or in crystalline forms. These aggregates in some cases cause gelatinization and in others do not. It is in the former group that we must suppose there exists a peculiar intensity of molecular attraction between the solid or internal phase and the water. As the name indicates all hydrophilic colloids exhibit this attraction for water and show a corresponding degree of viscosity so that as Höber expresses it the gels are not essentially different from hydrophilic colloid solutions. But in gels such as that formed by the fibrin-aggregates we must recognize a special degree in this attraction for water. The radius of the sphere of molecular action is large. The difference in this respect is illustrated especially well by fibrinogen solutions. When precipitated by acids or in other ways fibrinogen-aggregates are formed which eventually form large flocculi and settle out as a precipitate. When precipitated by thrombin, fibrin aggregates are formed which bind the water and form a gel.

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The crystallization process and the nature of the reaction between fibrinogen and thrombin. The vectorial characteristic which causes the fibrin aggregates under normal conditions to assume the form of definite needles is dependent in some way upon an interaction between the thrombin and the fibrinogen. That is to say fibrinogen never aggregates out in this way except under the influence of thrombin and on the other hand even under the influence of thrombin the aggregation may not occur in crystalline forms if the fibrinogen is modified by increasing the hydroxyl-ion concentration. The way in which the thrombin and fibrinogen react is not known. According to the older view thrombin plays the part of a ferment or catalyst which initiates or accelerates a chemical change in the fibrinogen. But nothing is known regarding the difference in chemical structure, if any exits, between fibrinogen and fibrin. The evidence at hand would indicate on the contrary that thrombin does not act as an enzyme (14), but forms a compound with the fibrinogen. The process of formation of fibrin as seen under the ultramicroscope suggests that the needles are formed by a physical union of thrombin and fibrinogen particles, the whole process being one apparently of aggregation as in the case of the flocking due to precipitating reagents. If this is the case we should expect that the process might be reversed. That is to say conditions might arise under which the particles would be redispersed and the thrombin and fibrinogen be separated. As is well known thrombin can be obtained from thoroughly washed fibrin by digesting the latter in strong solutions of sodium chloride, and the fibrin when thus treated gives also a protein in solution which resembles fibrinogen in some respects, for example, in the temperature of heat coagulation. Hekma (15) claims to have shown that the fibrin-gel is reversible. An alkaline solution of the fibrin may be made to gel again by appropriate treatment, by the addition of acids for example. But since this result is obtained when the alkaline solutions are boiled it seems evident that the thrombin factor is excluded in the second gelatinization and the reversal that he describes is not a reversal of the process or processes which lead originally to the formation of the fibrin-gel. No fibrinogen is formed in his method of reversal.

The most significant fact brought out in this paper in regard to the formation of the fibrin-needles is the connection of the crystallization process with the reaction of the medium. With a certain degree of alkalinity of fibrinogen solutions no needles are formed although a gel may still be obtained with thrombin. With a stronger degree of alka-

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linity no visible gel can be detected after the addition of thrombin but a distinct increase in viscosity may be demonstrated. The cataphoresis experiments described above indicate that under appropriate conditions part of the fibrinogen may carry a positive charge and part may carry a negative charge. The former exhibits only the phenomenon of aggregation or agglutination under the influence of the thrombin, the latter may show gelatinization without any visible aggregation of particles. The negative or positive charge exhibited by fibrinogen may be explained most easily on the assumption that it is due to electrical adsorption of hydroxyl or hydrogen ions. It is known that fibrinogen responds readily to changes in concentration of the hydrogen and hydroxyl ions in the solution. The former tend to favor the rapidity of the reaction with thrombin, the latter have a reverse effect. Under the normal conditions that prevail in the blood the thrombin has a double effect on fibrinogen. It causes, in the first place, an aggregation of the fibrinogen particles to form the fibrin needles and in the second place it sets up the process of gelatinization or binding of the water phase. In the cataphoresis experiments described these two effects of the thrombin are separated in a measure, the fibrinogen driven to the positive pole exhibits one, while that carried to the negative pole exhibits the other. As a provisional hypothesis one might assume that in neutral or slightly alkaline media the fibrinogen adsorbs or binds both hydrogen and hydroxyl ions, that the presence of the former furnishes an essential conditions for the aggregation into needles which takes place under the influence of the thrombin, while the existence of the hydroxyl combination, in connection with the presence of thrombin, is necessary to the development of the water-binding properties of the fibrin-aggregates. Under conditions such as the addition of excess of alkali it may be supposed that the fibrinogen particles exist in a higher degree of dispersion and exhibit adsorption of hydroxyl ions alone, and under these conditions thrombin causes only gel formation or increased viscosity, but is not capable of aggregating the particles into visible masses, either amorphous or crystalline in form.

CONCLUSIONS

1. In the clotting of mammalian blood the fibrin is deposited as needles. The needles are formed separately by an aggregation of fibrinogen particles. They vary in length from 10 to 30 microns and

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form a close meshwork. The normal clot may be described as a crystalline gel.

2. A similar crystalline gel is formed in the normal clotting of the blood of other vertebrates. The blood of the invertebrates (crab) gives a structureless gel.

3. The fibrinogen of mammalian blood may be modified so that it gives a structureless instead of a crystalline gel with thrombin. The simplest method of effecting this modification is by increasing the alkalinity of the blood. If the increase in alkalinity passes a certain concentration addition of thrombin fails to cause gel-formation but may still produce a distinct increase in viscosity.

4. In media with the normal reaction of blood thrombin and prothrombin when submitted to cataphoresis exhibit a negative charge. Under the same conditions the fibrinogen in oxalated plasma shows mainly a negative charge, but frequently, especially when the plasma is nearly neutralized, a part of the fibrinogen exhibits a positive charge. The reaction to thrombin of the positively and negatively charged portions is different.

5. In the theoretical discussion it is shown that the ultramicroscopic picture of the fibrin-gel is not explicable in terms of a honeycomb theory, that is, of the inclusion of a liquid phase within solid septa. The gel-character or property is due, probably, not to surface tension in the liquid films between the needles, but to surface action of the fibrin-aggregates upon the water.

6. The vectorial characteristic of the fibrin-aggregates is connected with the reaction of the medium. The view is suggested that adsorption of both hydrogen and hydroxyl ions by the fibrinogen particles plays a determining part in the two effects of the action of thrombin, namely, the directive aggregation of the particles and the property of gelatinization.

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THE TENSION OF CARBON DIOXIDE AND THE PERCENTAGE SATURATION OF THE HAEMOGLOBIN IN THE VENOUS BLOOD AT REST AND AT WORK

The Regulation of the Circulation Rate

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I

Some observations on the minute volume of blood passing through the lungs of man at rest and at work were published from this laboratory a year ago and at about the same time a very exhaustive treatise on the same subject appeared from the Finsen Institute in Copenhagen. The author, Lindhard (1), gave curves of the blood-flow which agree very closely with those plotted by us. Very recently Newburgh and Means (2) have published similar observations on two other subjects, one of whom was normal and the other had a double aortic and double mitral disease. They state

> For the sake of comparison we have plotted the blood-flows of C. L. and J. H. M. in terms of oxygen absorption and have shown them together with those of Boothby. The curves for blood-flow of the three subjects are very nearly coincident, which we believe is a fact of considerable importance in that it shows that the increase in the blood-flow is governed by the same law in different individuals, and is especially interesting since one of the three individuals observed had a badly damaged heart.

In the original paper (3) we showed that it was possible to construct from the data there given the following curves: (1) the blood-flow per minute; (2) the pulse rate; (3) total ventilation; (4) volume of blood per pulse beat; (5) percentage saturation of the haemoglobin in the mixed venous blood; (6) alveolar carbon dioxide tension; (7) respiratory quotient; (8) tension of carbon dioxide in the venous blood, allowing for the influence of the percentage saturation of the haemoglobin with oxygen; (9) hydrogen ion concentration of the arterial blood; (10) the

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tension of oxygen in the venous blood, allowing for the total acidity of the blood; and finally (11) Henderson's oxygen pulse. These curves are reproduced in this paper in figure I.

Curve VIII in figure I, representing the tension of carbon dioxide in the venous blood, was plotted after making the proper correction for the influence of the percentage saturation of the haemoglobin with oxygen. It is, of course, possible to plot the carbon dioxide curve without making this correction. This would then represent the carbon dioxide tension of the blood if the haemoglobin were completely saturated with oxygen, as is the case when the carbon dioxide tension is determined by the direct experimental method (Curve XII).

If direct determinations of the uncorrected carbon dioxide tension and oxygen tension[1] are made for venous blood and found to agree with the calculated values, a very convincing proof would be presented to substantiate the data of the original paper and the method of calculation adopted for determining the secondary curves.

II

The method adopted by us for determining the carbon dioxide and oxygen tensions in the venous blood is the one recently described by Christiansen, Douglas, and Haldane (4) and consists in using the lungs as an aerotonometer on the principle introduced by Pflüger. The technic is as follows: After a maximal expiration a mixture of carbon dioxide and air enriched with oxygen is inhaled from a Krogh recording spirometer; the breath is held about five seconds and then an expiration made to the "Mittellage" to obtain the first alveolar air sample; then the breath is held as long as possible and a maximal expiration made at the end of which the second alveolar air sample is taken. If the inspired mixture is of such a composition that when diluted with the residual air the carbon dioxide tension or the oxygen tension is within 3 or 4 mm. of the actual venous tension, it is reasonable to assume that after holding the breath twenty seconds longer (at rest) the air in the lungs will be in final equilibrium with the carbon dioxide or oxygen tension in the venous blood.

The determination of the carbon dioxide tension. The percentage saturation of the haemoglobin with oxygen influences the carbon dioxide

[1] If the oxygen tension of the venous blood is known, the percentage saturation of the haemoglobin can be obtained from the dissociation curve of oxyhaemoglobin, making allowance for the total acidity of the blood.

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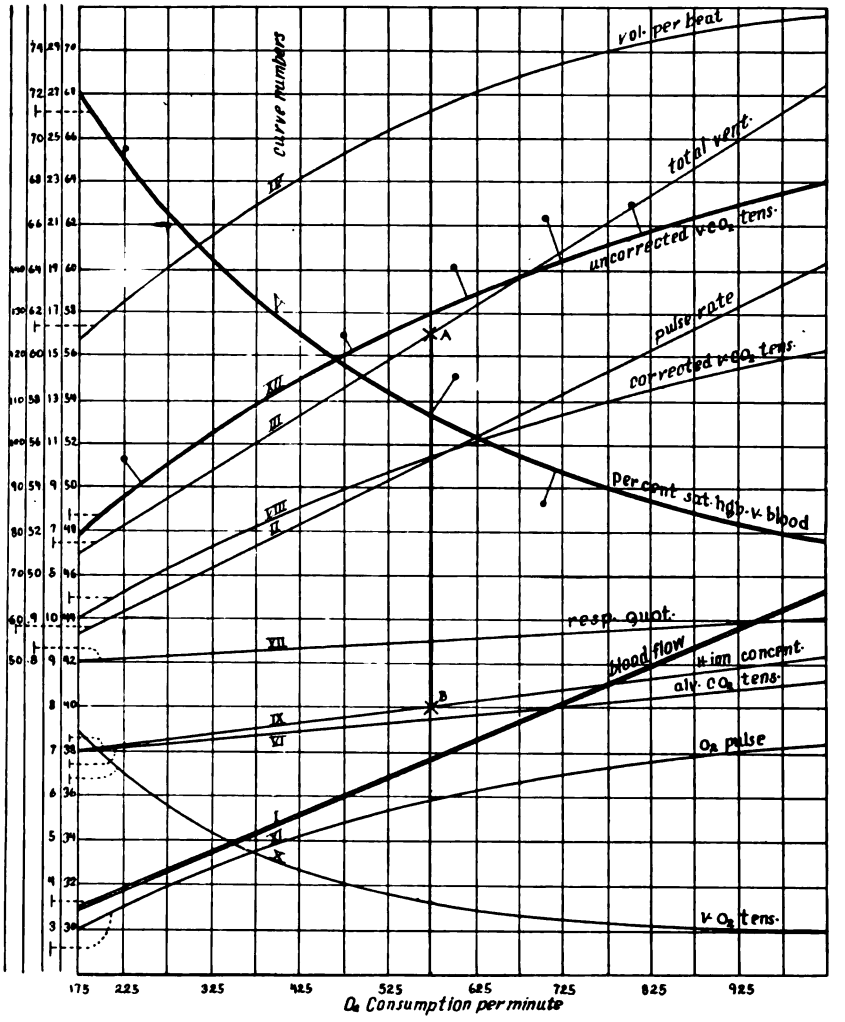


FIG. 1. Curves I to XI inclusive are the same as in our preceding paper (3). Curve I is the blood-flow per minute. Curve II, the pulse rate. Curve III, total ventilation per minute. Curve IV, volume per pulse beat. *Curve V, the percentage saturation of the haemoglobin in the mixed venous blood; connected to this curve are the points from Table I of this paper.* Curve VI, alveolar CO_2 tension. Curve VII, respiratory quotient. Curve VIII, tension of CO_2 in the venous blood, allowing for the influence of the percentage saturation of the haemoglobin with oxygen. Curve IX, hydrogen ion concentration of the arterial blood. Curve X, tension of oxygen in the venous blood allowing for the total acidity. Curve XI, oxygen pulse in cubic centimeters (Henderson). *Curve XII, uncorrected venous CO_2 tension determined from the nitrous oxide experiments; to this curve are connected the corresponding points given in Table I of this paper.*

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dissociation curve; consequently a large excess of oxygen must be present in the inspired mixture to insure complete saturation of the haemoglobin with oxygen. For experiments at rest a mixture was made containing about 0.5 liters carbon dioxide, 1.5 liters oxygen and 4.0 liters air. For experiments at work slightly more carbon dioxide was used corresponding to the degree of work. If the carbon dioxide percentage is too high, it is impossible to hold the breath for a sufficient length of time to establish equilibrium between the venous blood and the alveolar air; if too low, not enough carbon dioxide is brought back to the lungs by the blood during the experimental period to produce such an equilibrium; and finally, if the oxygen percentage in the lungs is too low, the haemoglobin will not be completely saturated. Therefore it is necessary to take great care to prepare a mixture in the spirometer that will, when diluted with the residual air, produce a carbon dioxide tension in the alveolar air within 2 or 3 mm. of the carbon dioxide tension in the venous blood.

The determination of the oxygen tension. The method of determining the oxygen tension is similar to that of the carbon dioxide tension, except that the inspired mixture must be made up with a very large proportion of nitrogen. For experiments at rest the dead space in the Krogh spirometer was washed out with nitrogen and from 0.5 to 1 liter of air introduced; nitrogen was then added making a total of 6 liters. For experiments at work pure nitrogen was used.

We have made a large number of determinations of the venous oxygen tension at rest and consequently feel that the accidental errors have practically disappeared in the final average. For the experiments at work the results are less satisfactory as breathing pure nitrogen was quite dangerous and therefore we performed only a limited number of experiments and were satisfied in obtaining the general trend of the curve.

Instead of plotting the venous oxygen tension directly we have transposed it into terms of percentage saturation of haemoglobin with oxygen by means of the dissociation curve of oxyhaemoglobin given in figure 4 of the previous paper. This latter form of expression makes allowance for the variation in the carbon dioxide tension in the blood due to the method of obtaining the oxygen tension and is, therefore, the better way of representing the amount of oxygen in the venous blood.

As the tension of carbon dioxide and the percentage saturation of the haemoglobin in the venous blood vary with the amount of oxygen absorption per minute, a condition of body equilibrium must be estab-

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lished. Experience has shown that this requires a preliminary period of about one-half hour during which time the subject rests or works at the same level as in the experiment proper. To obtain the oxygen consumption per minute a complete respiratory exchange experiment precedes the experiment proper.

For details of experimentation and calculation of results omitted in this communication, the reader is referred to our earlier paper.

III

In all we have performed two hundred and twelve experiments on the direct determination of the uncorrected carbon dioxide and oxygen tensions of the venous blood at rest and at work. The results are averaged into groups and presented in condensed form in the following table—Table I.

TABLE I

OXYGEN ABSORPTION	AVERAGE UNCORRECTED VENOUS CO_2 TENSION	NUMBER EXPERIMENTS IN AVERAGE	AVERAGE DEVIATION FROM MEAN	PERCENTAGE SATURATION HAEMOGLOBIN	NUMBER EXPERIMENTS IN AVERAGE	AVERAGE DEVIATION FROM MEAN
cc.	*mm.*		*mm.*	*per cent*		*per cent*
225	51.5	68	1.8	69.5	89	2.6
473	57.0	12	1.8			
604	60.1	8	3.1	59.3	6	3.8
705	62.4	10	4.4	53.4	13	7.6
800	63.1	6	1.9			

The average figures given in Table I are plotted in figure I. The large black dots represent the points determined by the present experiments and they are connected by a line to the corresponding curve calculated from the data of the previous investigation.

The direct determinations of the uncorrected venous carbon dioxide tension are seen to fall on a line parallel to but from 1.25 to 1.75 mm. higher than the curve calculated from the data of the previous experiments (Curve XII).

As these points stand they are an exceedingly strong confirmation of the previous data and calculations. The slight discrepancy in the two values is, on close examination, a further confirmation of the accuracy of the methods. In both there is a constant error due to the assumption that no blood makes a complete circuit during the time of the experiment. It was pointed out in the previous paper that the con-

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stant error due to the presence in the lungs of blood that was making its second circuit was of an unknown but probably very slight order. By the nitrous oxide method the error from this assumption will cause the calculated tension to be slightly too low; in the present series, the same constant error will cause the tension determined directly to be correspondingly too high. Therefore, the true uncorrected venous carbon dioxide tension will be a mean between the curve calculated from the nitrous oxide experiments and that drawn through the points presented in this paper. The order of the error due to the recirculation of part of the blood during the time of an experiment is, then, in the region of 0.75 mm. for the uncorrected carbon dioxide tension.

The point indicating the percentage saturation of the haemoglobin with oxygen in the venous blood at rest represents the average of eighty-nine experiments. It lies within 0.5 per cent of the curve calculated from the nitrous oxide experiments and is therefore another strong confirmation of the accuracy of both methods.

The two averages obtained for the percentage saturation of the haemoglobin at work were from only a few experiments. They fall, however, about 2 per cent from the calculated curve, one above and the other below it. They show the trend of the curve but do not establish its position with the exactness of the uncorrected venous carbon dioxide curve (Curve V).

As pointed out by Christiansen, Douglas, and Haldane, it is possible from the above data to calculate the volume of blood passing through the lungs and it is obvious that essentially the same results would be obtained from these experiments as we found by the nitrous oxide method.

The fact that there is such close agreement in the data obtained by two entirely different methods is a very convincing proof of the accuracy of both methods for determining and calculating the circulation rate, the carbon dioxide tension and the percentage saturation of the haemoglobin in the venous blood.

It is evident therefore, that the circulation rate increases under conditions of work with the oxygen consumption in a manner corresponding to the increase in the total ventilation. We previously suggested that the actual activating substance which increases both the circulation rate and the total ventilation is the hydrogen ion concentration of the arterial blood. The fact, however, that an increase in the total ventilation can be very great when breathing carbon dioxide at rest without a corresponding increase in the circulation rate, as evidenced

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by a proportionate increase in the pulse rate, suggests the possibility that the ventilation center is more sensitive to an increase in the carbon dioxide component and the circulation center to an increase in the non-volatile acid radicals composing the total acidity.

SUMMARY

1. The results are reported of two hundred and twelve experiments on the direct determination of the uncorrected carbon dioxide tension and percentage saturation of the haemoglobin in the venous blood at rest and at work.

2. The averages for various oxygen consumptions of the uncorrected venous carbon dioxide tensions fall on a line parallel to but from 1.25 to 1.75 mm. higher than the curve calculated from the experimental data obtained in determinations of the circulation rate by the nitrous oxide method, previously reported by Boothby.

3. The discrepancy in the two curves is caused by the error from the recirculation of the blood working in opposite directions in the two methods.

4. The average of the determinations of the percentage saturation of the haemoglobin in the venous blood at rest lies within 0.5 per cent of the curve calculated from the nitrous oxide experiments. One of the two averages for the percentage saturation at work falls 2 per cent above and the other 2 per cent below the calculated curve.

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ON THE DETERMINATION OF CHARACTER AND QUANTITY OF THE RESPIRATORY CHANGE OF ARTERIAL PRESSURE IN MAN BY MEANS OF THE KOROTKOFF SOUNDS

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Among the questions that may be answered from the data to be had from routine indirect arterial blood pressure determinations on man there are two of considerable interest. These are (1) What is the character of blood pressure change during a respiration, does the pressure rise or fall during inspiration? (2) How much in terms of mm. of Hg. may the pressure be made to rise and fall during a respiration?

If Erlanger's sphygmomanometer is used the answer to the first question may be given by simultaneous observation of the cardio-respiratory waves in the sphygmomanometer tracing and the breathing movements (1). To do this well, however, a simultaneous tracing of the respiratory movements should also be taken as shown by Erlanger and Festerling (2) and as one of the present authors did in an extension of that work (8). Lewis (7) used a somewhat different method to determine the phase of respiratory rise of pressure, namely, by noting the phase of respiration during which the pulse-waves pass through the radial artery when a high systolic pressure is applied to the brachial artery. For this a "suspended sphygmograph" was used over the wrist.

In what immediately follows it will be shown that the Korotkoff sounds alone may be used for the determination of the first question put above. It will then be shown that by means of the Korotkoff sounds one may also measure the extent of blood pressure oscillation during the respirations.

During a study "on the inversion of the respiratory wave" in the blood pressure trace of man (8) it was observed that the Korotkoff sound when the cuff pressure was set in the vicinity of systolic or diastolic pressure is not heard with every pulse-wave. The sound seemed to become audible for only a part of the respiration, if one noticed the

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respiratory movements. By having the subject breathe deeply and slowly the intermittent character of the sounds became so pronounced that the phenomenon was observed without difficulty. A practiced ear, however, could note the phenomenon even when the subject breathed normally.

A systematic exploration of this periodicity of the Korotkoff sounds soon showed that if the cuff-pressure was gradually lowered from systolic toward mean blood-pressure the period of silence during the respiratory cycle grew correspondingly shorter and the period of sounds correspondingly longer until a sound was heard (as usually observed) for every pulse-beat of the whole respiratory act.

It was recognized that this periodicity of the Korotkoff sounds is due to the fact that the levels of systolic and diastolic pressure themselves ebb and flow within the interval of a respiration.[1] And so with cuff-pressure set at mean systolic pressure a sound ought to be heard for every pulse-beat only during that portion of the respiration in which the (internal) systolic pressure is equal to or more than the (external) cuff-pressure. On the other hand, during that part of the respiration alone in which the (internal) systolic pressure is less than the (external) cuff-pressure the pulse-beats would be unaccompanied by sounds.

Furthermore under these conditions, and given that the inspirations are equal in time with the expirations, the period of sounds and the period of silence ought each to cover about one-half of the whole respiratory cycle. As the cuff-pressure is set above or below mean systolic pressure the ratios of the time intervals of sounds and silence ought to decrease or increase correspondingly, until the one or the other entirely vanishes.

If this explanation is correct for the periodicity of sounds with cuff-pressure in the region of systolic pressure then also one ought to observe for similar reasons, it was argued, a periodicity of sounds with cuff-pressure set in the region of diastolic pressure. Only in this case sounds would be heard with pulse-beats during that part of respiration alone in which the (internal) diastolic pressure falls below the (external) cuff-pressure; failure of sounds would cover that period of the respiration only during which the diastolic pressure remains above the level of cuff-pressure. With the latter again set at mean diastolic pres-

[1] By means of a sphygmograph attached to the wrist Lewis observed a similar periodicity of pulse-waves reaching the radial artery, when cuff-pressure was at systolic or high systolic pressure.

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sure the two periods, one of sounds and one of silence, during a single respiration ought to be of equal duration.

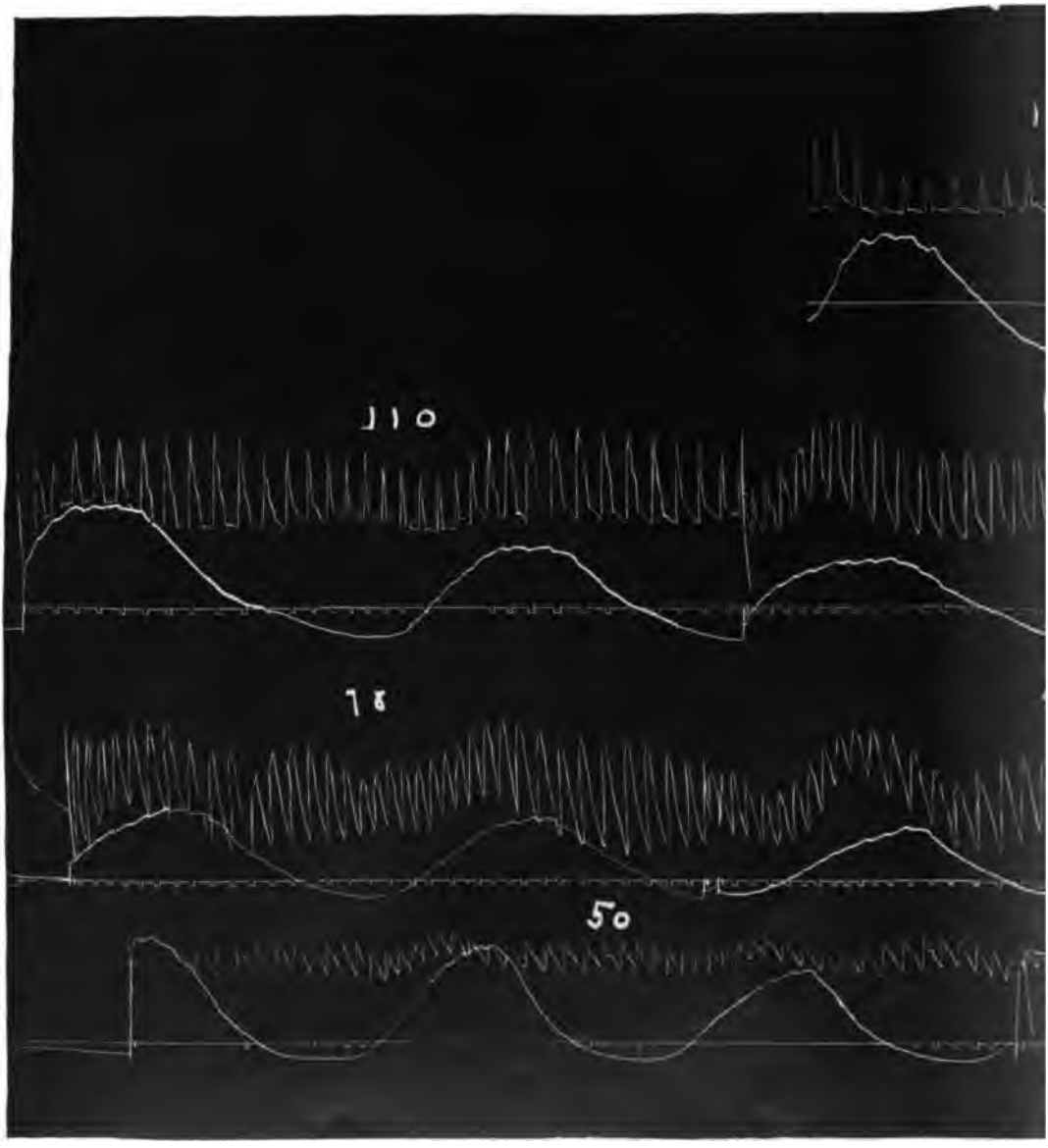
While the periodicity of sounds in the two critical regions of blood pressure may thus yield pictures of similar character, there will be a common feature of striking difference. This difference ought to be one of right and left-handedness, or the difference of an object and its reflected image. With cuff-pressure at level of diastolic pressure the period of sounds ought to fall just in that part of the respiratory cycle which, when cuff-pressure is at level of systolic pressure, is covered by the period of silence, and *vice versa*.

Experiment showed that this is the case, as will be seen in the record below (fig. 1), and it may be pointed out here that this reversal of the periodicity of Korotkoff sounds in relation to the two phases of the respiratory act is only another expression of the "inversion of the respiratory wave in the blood pressure trace of man" to which reference has already been made (8).

THE DETERMINATION OF THE CHARACTER OF THE RESPIRATORY CHANGE OF PRESSURE LEVELS

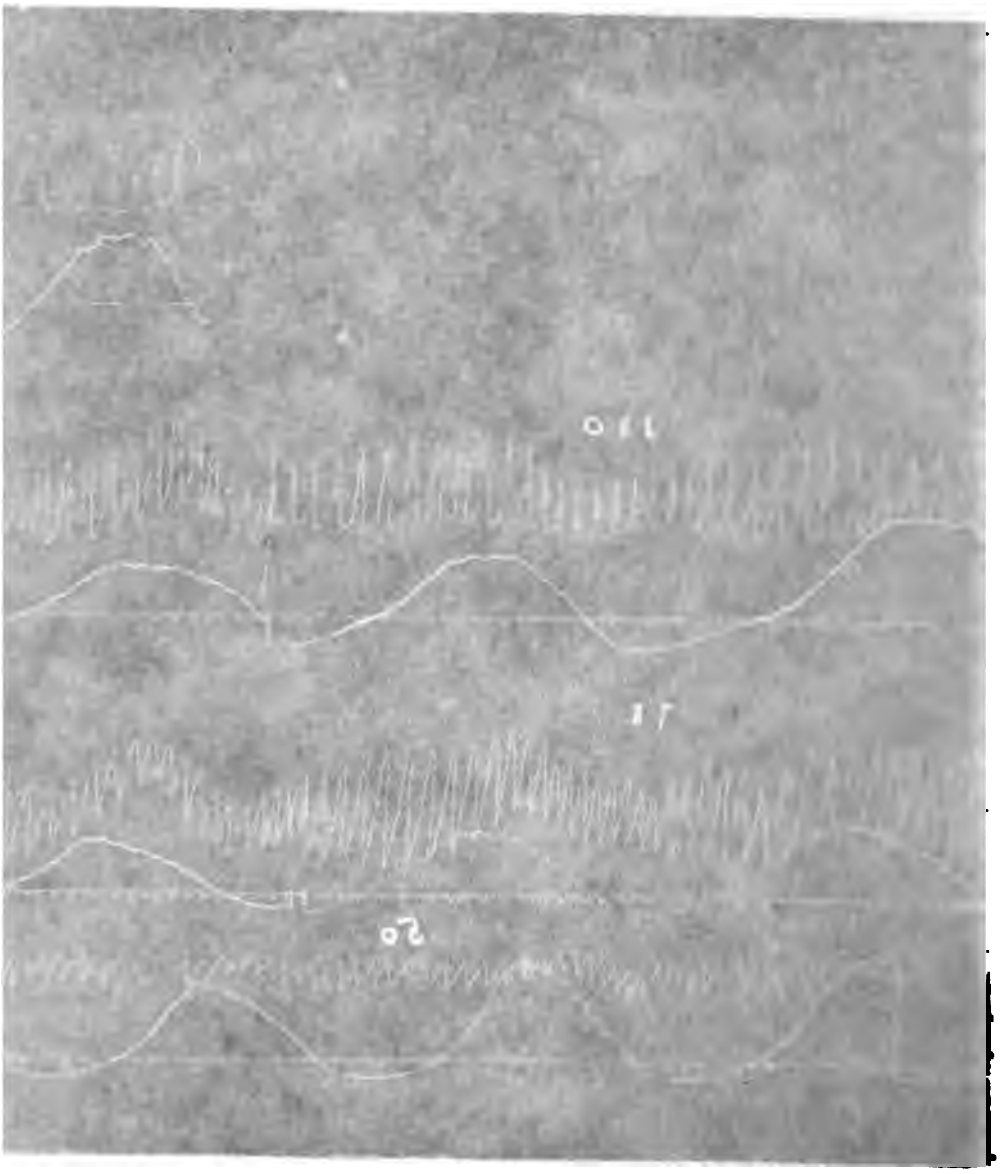
It becomes clear now that we have a simple and direct means of determining whether rise of blood pressure accompanies the inspiratory or the expiratory phase of respiration. One need only set the cuff-pressure in the region of mean systolic pressure, have the subject breathe deeply and slowly, and observe simultaneously the periodic sounds and the phases of the respiration. If the period of sounds is heard during or at end of inspiration, or the period of silence occurs during or at end of expiration, the subject has *inspiratory* rise and expiratory fall of pressure. On the other hand, if the period of silence falls in with the act of inspiration and the period of sounds with the act of expiration, the subject has clearly an *expiratory rise* and inspiratory fall of arterial pressure. It may be said at once that we have observed both these kinds of respiratory change of pressure. The cardio-respiratory wave characterized by inspiratory rise, however, has been the more common among our cases, mostly young men. It has further been observed that the cases of inspiratory rise are also cases of inspiratory acceleration of heart-rate and apparently fall among the type showing the "young-heart" of Mackenzie, that is, those having labile vagal centres as discussed by one of us in an earlier paper (5).

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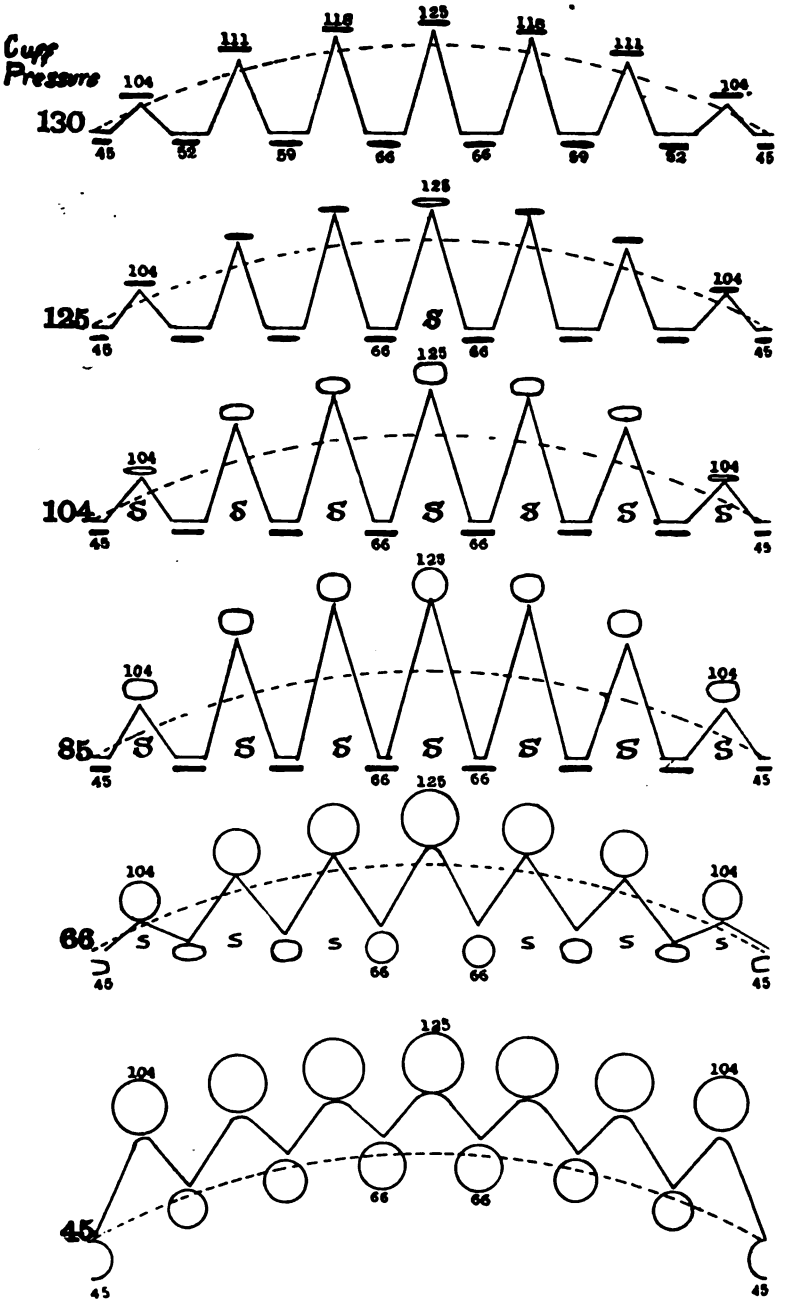
THE RELATION OF THE KOROTKOFF SOUNDS TO THE CRITICAL BLOOD PRESSURES IN MAN

As the reader doubtless has noted already, the principal argument in this paper depends upon the correctness of the view that the first and last Korotkoff sounds are true indices of systolic and diastolic pressures. As to the systolic index there is general agreement. That the last sound indicates diastolic pressure, however, is still in dispute. Since the *identity* of the first and last sounds with the critical pressures is the major premise in the argument, it makes it necessary for us to state what views we hold as to the conditions under which the sounds are produced. Inasmuch as other authors have already begun a systematic study of the causes of these sounds,[2] we shall make our discussion as brief as possible. One need not discuss the ultimate physical causes of the sounds heard over the compressed artery. The "water hammer" effect of Erlanger (5) appears to be very plausible and there seems to be very good ground for the necessary "half flattening" of the artery as brought forth by MacWilliams and Melvin (10). The views advanced by all these authors are freely drawn upon in what follows.

Evidence that the artery is completely flattened is obtained (if one has a delicate instrument) from the sphygmomanometer tracing itself. This becomes clear from the following consideration: In the ordinary sphygmomanometer tracing the individual pulse waves are made up of an upward excursion of the writing lever with systole due to an increasing arterial volume and a downward excursion with diastole due to a decreasing volume. Should the external pressure upon the artery be sufficient to cause it to collapse during a diastolic phase, a further fall of pressure in the artery (in more complete diastole) can impart no further change in volume to the cuff, for the volume of the compressed artery is already at a minimum. Therefore when the artery collapses for a certain period in the diastole one ought to find it indicated upon the tracing by a horizontal line marking the trough (diastole) of the pulse wave. For this horizontal line must indicate a constant arterial minimal volume and can be due only to obliteration of the artery. Otherwise the constantly changing arterial volume would make it impossible for the lever to remain at the same level for a period of time. In the same way when no horizontal is found in the trough of the pulse wave no constant volume is struck in diastole and the ar-

[2] See principally Erlanger (4, 5) and Brooks and Luckhardt (6).

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Explanatory note to figure 2

1 The diagram represents the principal blood-pressure events occurring within six respiratory cycles during a deep breathing (decompression) experiment.

2. Each cycle is shown with cuff pressure at a different level of pressure, the amount of which is indicated in the large numerals along the left of the diagram.

3. Each respiratory cycle moreover contains seven smaller waves representing the pulse waves of a sphygmomanometer trace, the crests of the waves being heights of systole, the troughs depths of diastole. The long dotted line indicates phases of the respiration, the upstroke being inspiration.

4. At the crest of each systole is figured the relative form and size of a cross section of the artery under the cuff for that particular systole; below the trough is figured the artery's lumen for that particular diastole. If complete obliteration obtains the lumen is indicated merely by a heavy dash.

5. The small numerals along the crests of the pulse-waves indicate the internal arterial pressure at height of the corresponding systoles. The small numerals below the troughs of these waves indicate corresponding diastolic pressures.

6. The pulse waves producing Korotkoff sounds are so indicated by a letter *S*, a large letter for the louder sounds, a small letter for the softer sounds.

7. At 130 mm. cuff pressure no sounds occur and complete obliteration of the artery prevails.

At 125 mm. cuff pressure one sound only is heard and the first patent lumen appears—"period of sounds."

At 104 mm. cuff pressure all pulse waves produce sounds and the lumens alternate between complete and partially flattened contours.

At 85 mm. cuff pressure all diastoles have obliterated lumens and one systole is able to change the lumen to a full circular shape.

At 66 mm. cuff pressure the first sound drops out—"period of silence." Other pulse waves produce the softer sounds, the lumens alternating between circular and partially flattened contours. The wave failing to produce a sound has the lumens alternating between contours of circles only, a condition which prevails for all pulse waves, with total absence of sounds, when cuff pressure is lowered to 45 mm. pressure.

8. In the sections of the diagram with cuff pressure set at 104 mm. Hg. or less an attempt is made to indicate roughly the portion of diastolic and systolic period during which the artery is completely obliterated. This is done by varying the length of the horizontal line in the trough of the pulse wave. As will be seen the flat troughs disappear entirely with cuff pressure as low as 66 and 45. Had this plan been carried out in the scheme with cuff pressures at 130 and 125 the pulse waves would all, save one, be perpendicular with the two limbs of each wave superimposed and the flat troughs covering the whole of the systole as well as diastole. These changes in the flattening of trough in the pulse waves can well be seen in sphygmomamometer tracings.

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tery must not have been collapsed at any time during the cardiac cycle in question.

We are not to infer that when the external pressure is insufficient to cause collapse of the artery there is no deformation of the arterial cross section during diastole. It has been shown (MacWilliams and Melvin) that the artery undergoes a phase of partial flattening with lowered external pressures. Finally the external pressure may be lowered to such an extent that there is no deformation of the arterial crosssection even during complete diastole. Under these conditions the artery is in the same condition as regards its contour (but not as regards its diameter) as it would be were the cuff and external pressure absent altogether.

By inspection of the record (fig. 1) this sequence of events in regard to the character of the pulse wave is seen graphically recorded. At the pressure 85 mm. Hg. it is seen that the pulse tracing coincident with the lowered arterial pressure during expiration (down stroke of respiration lever) has flattened troughs (diastole), while with the higher arterial pressure during inspiration there is no flattening of the troughs. In the first case the artery has collapsed during diastole, in the second case it has remained patent for the whole cardiac cycle. Contrasting this region of the record with that at 120 mm. Hg. external pressure and that at 71 mm. Hg. external pressure it is seen that in the former the artery collapses with each and every diastole, while in the latter the record indicates that it is patent during the whole cardiac cycle and we infer (since a sound is produced) that it suffers only a certain degree of flattening. It was observed in our experiments indeed that the change from the phase of collapse to the phase in which collapse does not occur is marked by a change in the character of the sounds heard. A schematic representation of events as they occur in this record together with their relation to sound production has been prepared in figure 2. The explanatory note describes it in detail.

On the basis of the water hammer theory of sound production (Erlanger) one finds an explanation of the changing character of the sounds (as external pressure is lowered) which is most suggestive and adds something to our notion of their significance and value as criteria. As external pressures are lowered below the systolic level, the intensity of the sound increases to a certain maximum of intensity and then *suddenly* diminishes. From this point on the sounds become fainter and finally disappear. One should be able to explain the changing intensity by a corresponding change in water-hammer-pressure. The evi-

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dence of changing water-hammer pressure can be found in the character of the pulse wave (length of obliteration period), while the changes in sound intensity may be observed and the relation of the one to the other noted.

If during the cardiac cycle the artery underneath the cuff becomes obliterated, the column of blood distal to the cuff will become practically motionless (being moved only by contraction of the walls of the distal segment), since no blood can pass the obliterated segment and enter it from above. Moreover the degree to which this distal column of blood is slowed cannot be greatly influenced by the length of time during which obliteration lasts. Once arrested by obliteration, continued obliteration can add little to the effect. Under these cirumstances one factor in the production of a water hammer pressure is at its maximum. On the other hand, the obliterated segment offers a resistance to the pulse wave proceeding through the proximal segment and serves to reduce its force and velocity before its impact upon the distal segment occurs. Though the length of the obliteration period does not materially effect the velocity of the distal column, yet as the period is shortened there will be a corresponding increase in the force and velocity with which the proximal column strikes the distal column (compare Erlanger (5), p. 86). Therefore as the obliteration period is shortened with the lower external pressure there is an increase of water hammer pressure with each lowering of external pressure. This increase should continue to the point at which obliteration fails to occur. When the external pressure is lowered to a point at which no obliteration occurs, the distal column of blood is kept moving throughout diastole, for blood is allowed to pass the segment underneath the cuff and enter the distal artery. The impact of the proximal column upon this moving distal column will result in a decreased water hammer pressure being produced. The transition from a stagnant to a moving distal column is sudden, and there is a correspondingly sudden decrease in water hammer pressure. Also we should expect at this point a sudden decrease in the intensity of sound. The relation of the obliteration period and its effect on the factors producing a water hammer pressure as we conceive it, is shown in the diagram (fig. 3).

Evidence of this sequence of events together with its relation to sound production is obtained from the record in figure 1. Changes in the water hammer pressure may be followed by the reader, as it was observed in our experiments, by the changing length of time during which the trough of the pulse wave runs horizontally. The horizontals

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(in trough of pulse wave) indicating obliteration decrease in length as the sound becomes more intense to the observer. The disappearance of these horizontals indicating no obliteration was attended by the sudden change in the intensity of the sound. Obviously the external pressure sufficient to cause obliteration is somewhat above the lowest (diastolic) pressure occurring in the artery during a cardiac cycle,

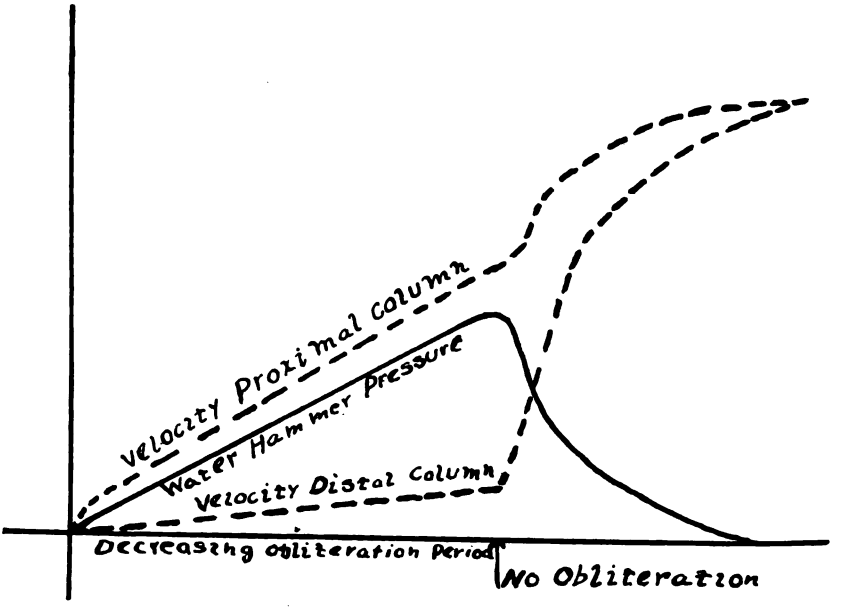


Figure 3

hence the change of sounds is not to be taken as an index of diastolic pressure. From this point down sounds continue. A point is finally reached where no sound is produced. We are led to believe by inference that this lower period of sound production is attended by some deformation of the arterial wall. The level of external pressure at which no deformation occurs should be equal to the diastolic pressure. At this level the internal and external pressures simply equalize each other.

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THE QUANTITATIVE DETERMINATION OF THE RESPIRATORY CHANGE OF ARTERIAL PRESSURE

As stated in an earlier section the Korotkoff sounds may also be used as an index in the measurement of the extent of blood pressure change accompanying respirations.

During a decompression experiment the level at which the first few sounds are heard (or at which the period of sounds is first introduced) must be the level of the highest systolic pressure during a respiratory cycle. This may be called the *maximum respiratory systolic pressure.*

When upon further decompression the sounds are constant for the first time throughout the whole of the respiratory cycle, the level of the lowest systolic pressure is reached. This pressure may be called the *minimum respiratory systolic pressure.*

Similarly when further decompression brings one into the diastolic region, there will be a transition from constant sounds during the whole of respiration to a level where the sounds begin to take on a periodic character again. The pressure at which this second periodicity of sounds just begins is taken as the highest diastolic level and has been called the *maximum respiratory diastolic pressure.*

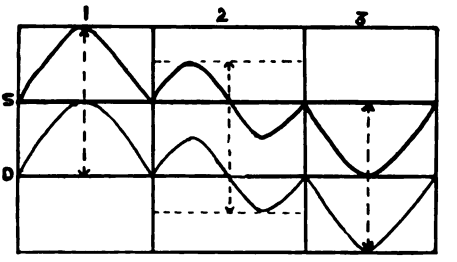


Figure 4

Upon still further decompression even the periodic sounds drop out. The level of pressure at which the period of silence for the first time covers the whole of a respiratory cycle is taken as the lowest diastolic pressure during a respiration and has been called the *minimum respiratory diastolic pressure.*

It should be stated at this point that the directions in which deep respirations influence the maximum and minimum systolic and diastolic levels away from what would be (mean) systolic and diastolic pressures with quiet breathing may be various. This is graphically shown in figure 4, where three cases are plotted.

(1) The maximum systolic is raised above the systolic level of quiet breathing, while the systolic level of quiet breathing now becomes the minimum systolic.

(2) In addition to the raising of the maximum systolic above the

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level of normal breathing, there is a fall of the minimum systolic below this level.

(3) The systolic level of normal breathing becomes the maximum systolic while the minimum is reduced to a point below the systolic level of normal breathing.

The nature of the change in the diastolic level is the same as that in the systolic level. These combinations are put to schemata in the figure 4. Probably in the usual case something resembling (2) is what actually occurs—a combination of (1) and (3).

In any case the "Respiration pulse pressure" (heavy broken lines) far exceeds the ordinary pulse pressure (distance from S to D, the systolic and diastolic levels of quiet breathing).

METHOD AND RESULTS

The usual wide-cuff compression bag was applied to the arm enclosing the brachial artery. In the path of the compression chamber were inserted the usual mercury manometer, inflation bulb, needle valve and Marey sphygmoscope. The latter connected directly with a double lever Marey tambour. Instead of the parts of the apparatus being crowded together compactly as is usual in a sphygmomanometer for bedside use, they were strung out in a linear arrangement with the sphygmomanometer lever at one end free to be applied to the writing surface of an ordinary drum kymagraphion.

Provision was made for simultaneous tracings on the drum record for the following:

(a) The blood-pressure, or sphygmomanometer lever.

(b) The mechanical respiratory movements. A lever with Ludwig writing tip (chord recording lever) traced these movements. The transmission of the movement was done by a pneumatic system of elastic bags, with a Marey bulb intercalated.

(c) An electro-magnet signal lever to record sounds. The key of this lever was operated in the hand of the person listening for the Korotkoff sounds. Every sound was thus recorded throughout an experiment. The loss of time (mechanical and reaction time of the observer) in recording the separate sounds was neglible in this work because we were not trying to determine at what point of time in the cardiac cycle the sound was produced. The method of stopping the drum at each change of level of cuff pressure automatically[3] marked off on the record the portion belonging to each of the pressure levels.

[3] By means of the scratch marks of the moving lever tips. Thus also does one have a continuous control of the vertical relation of the writing tips.

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In case continuous decompression was elected

(d) a fourth signal lever operated by a key in the hand of another observer marked off each 5 mm. fall in manometer pressure. This was only employed in continuous decompression experiments. In most of the experiments the subjects were required to breathe as deep and slowly as they could with comfort for the duration of an experiment.

A type of the graphic records thus obtained appears in figure 1, scrutiny of which will readily show:

(a) The periodicity of the Korotkoff sounds in the regions of systolic and diastolic pressures.

(b) The inversion, or reversal, of the position of the "period of sounds" in relation to the "period of silence" of a respiratory cycle in the systolic region when compared with a respiratory cycle in the diastolic region.

(c) The inversion of the respiratory wave in the sphygmomanometer trace as described in a previous communication.

(d) The levels at which maximum respiratory systolic pressure occurs—shortest period of sounds, and minimum respiratory systolic pressure—shortest period of silence, or transition from periodic to constant sounds, both in the systolic region of the sphygmomanometer trace.

(e) The levels at which the maximum and minimum respiratory diastolic pressures occur—maximum and minimum periods of sounds in the region of diastolic pressure.

(f) The inspiratory rise and expiratory fall of blood pressure is shown by position of the "period of sounds" in relation to the phases of the respiration. See trace of respiratory movements. Upstroke of lever is the inspiratory movement.

(g) The close correspondence between the periodicity of the sounds and the rise and fall of the cardio-respiratory wave in the sphygmomanometer trace.

(h) The relation of changing heart rate to rise and fall of blood pressure.

Most of the experiments were intermittent decompression experiments, the pressure intervals being 5 mm. The order of events therefore is the order as they occur with cuff-pressure passing from higher to lower manometer levels. And this also is, as far as the data are concerned, the order adopted in recording the results as shown in table 1.

Stress for a long time has been laid upon the importance of pulse pressure in the normal experimental perfusion of the various organs of the

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TABLE 1

Deep breathing experiments

NUMBER OF EXPERIMENT		RESPIRATORY SYSTOLIC PRESSURES		RESPIRATORY DIASTOLIC PRESSURES		RESPIRATORY PRESSURE RANGES	
		Maximum	Minimum	Maximum	Minimum	Systolic	Diastolic
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
I	1	133	103	68	55	30	13
	2	138	103	50	30	35	20
	3	145	110	95	60	35	35
	4	130	110	76	62	20	14
	5	125	115	68	55	10	13
	6	140	112	90	74	28	16
	7	125	104	66	45	21	21
II	8	125	80	50	35	45	15
	9	140	100	60	50	40	10
	10	130	110	70	55	20	15
	11	115	90	60	36	25	24
	12	126	90	60	36	36	24
	13	130	85	60	40	45	20
	14	135	110	62	50	20	8
III	15	134	105	65	40	29	25
	16	140	120	72	50	20	22
IV	17	100	80	65	50	20	15
V	18	130	95	81	78	35	3
	19	128	106	95	76	22	19
VI	20	133	113	100	85	20	15
VII	21	125	98	60	50	27	10

Explanatory note to Table 1

Column 1. The number of experiment is merely here indicated. Data concerning the conditions of the experiment will be found in Table 2 after the corresponding numbers. The Roman numerals refer to different subjects.

Column 2. The level of cuff-pressure at the moment when the periodic sounds first were heard are here listed. The level is styled the *maximum respiratory systolic pressure.*

Column 3. The level of cuff pressure at the moment when the sounds lose their periodicity with reference to the respiration is noted in this column. The pressure is styled the *minimum respiratory systolic pressure.*

Column 4. The level of cuff-pressure at the moment when, during further decompression the sounds again first fail to be heard for a portion of the respiration interval. This pressure is called the *maximum respiratory diastolic.*

Column 5. The level of cuff-pressure when the period of sounds becomes reduced to one or two pulse beats per respiration, or the level at which for the first time no sounds are heard at all during a respiration. Styled, *minimum respiratory diastolic pressure.*

From the observed data contained in these four columns may now be calculated the following:

Column 6. The difference between maximum and minimum respiratory systolic pressures. This gives the *respiratory systolic range.*

Column 7. The difference between the maximum and minimum respiratory diastolic give the *respiratory diastolic range.*

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body, in physical examinations, and in clinical diagnosis of pathologic conditions (2). Pulse pressure is indicative of a combination of well known factors, such as elasticity of arterial bed, rate and force of heart-beat, etc.

The act of respiration may throw into the whole circulatory system another oscillation wave of equally great magnitude, synchronous not with the heart-beat, but with the respiration itself. Upon this wave the more frequent oscillations of the heart-beat are superimposed. To condense the picture of the cardiac effect in a single term the earlier authors chose the expression, pulse-pressure. To condense the picture of the respiratory effect in a single term one may similarly speak of the *respiratory-pressure.* This would be the difference between the two systolic levels or diastolic levels, or the effect as expressed by the terms (see columns 6 and 7 of table 1) respiratory systolic or respiratory diastolic range.

The combined effect of these two great oscillatory changes, pulse pressure and respiration pressure, with all their underlying physiological factors may likewise, therefore, be summed up in the term, *Respiration-Pulse-Pressure.*

The data for the calculation of this pressure will likewise be found in table 1. From the maximum respiratory systolic one simply subtracts the minimum respiratory diastolic pressure. This has been taken as the measure of respiration-pulse-pressure, for obviously the "maximum respiratory systolic" would not have been so high nor probably the "minimum respiratory diastolic" so low, but for the act of deep respiration.

The respiration-pulse-pressure as thus measured from our data are shown in table 2. Here also are added other data belonging to the experiments which for convenience of printing do not appear in table 1. The mean pulse pressure for each case is also added for comparison. The data for the mean pulse pressure will be found in table 3.

Determinations of the mean pulse pressure (and of both systolic and diastolic pressures) should have been made while the subjects were breathing shallow or holding the breath in each experiment for controls. This will be done in the work to follow. The increase in pressure changes of the deeper breathing compared with the normal breathing may be even more striking than the increase shown in table 2. Be that as it may, if one takes the ratios of the increase of respiration-pulse-pressure over and above that of the corresponding mean pulse pressure for the deep breathing experiments as shown in table 2, one

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TABLE 2*

NUMBER OF EXPERIMENT		SUBJECT	DATE	CHARACTER OF BREATHING	RESPIRATION PULSE PRESSURE	ORDINARY PULSE PRESSURE
I	1	R. C.	11/5/15	Abdominal-thoracic	78	57
	2	R. C.		Abdominal-thoracic	108	80
	3	R. C.	18/5/15	Abdominal-thoracic	85	50
	4	R. C.	3/12/15	Abdominal	68	51
	5	R. C.	3/12/15	Thoracic	70	59
	6	R. C.	15/12/15	Abdominal	66	44
	7	R. C.	26/2/16	Thoracic	80	59
II	8	F. F.	4/5/15	Abdominal-thoracic	90	60
	9	F. F.	18/5/15	Abdominal-thoracic	90	65
	10	F. F.	18/5/15	Abdominal-thoracic	85	58
	11	F. F.	1/12/15	Thoracic	79	54
	12	F. F.	1/12/15	Abdominal	90	60
	13	F. F.	1/12/15	Abdominal	90	57
	14	F. F.	26/2/16	Thoracic	85	68
III	15	J. G. H.	18/5/15	Abdominal-thoracic	94	67
	16	J. G. H.	18/5/15	Abdominal-thoracic	90	69
IV	17	L. K.	1/12/15	Thoracic	50	33
V	18	C. S.	4/12/15	Thoracic	52	34
	19	C. S.	4/12/15	Abdominal	52	42
VI	20	J. H.	18/8/15	Abdominal-thoracic	48	31

* See tables 1 and 3 for the data from which these pressures are determined.

TABLE

Mean arterial pressures in deep-breathing experiments in mm. of mercury

NUMBER OF EXPERIMENT		SYSTOLIC	DIASTOLIC	PULSE PRESSURE*
I	1	118	61	57
	2	120	40	80
	3	127	77	50
	4	120	69	51
	5	120	61	59
	6	126	82	44
	7	114	55	59
II	8	102	42	60
	9	120	55	65
	10	120	62	58
	11	102	42	54
	12	108	48	60
	13	107	50	57
	14	122	54	68
III	15	120	53	67
	16	130	61	69

* This pressure is obtained from the difference of the mean systolic and mean diastolic. Practically, though not invariably, the same figures are obtained by taking the difference between maximum systolic and maximum diastolic or between minimum systolic and minimum diastolic pressures.

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finds the increase to vary from 19 per cent to 58 per cent. The average of all the percentages is very nearly 40 per cent.

GENERAL REMARKS

Since the magnitude of respiratory pressure varies with the algebraic sum of the respiration factors it at first did not appear that there could be any constancy in the numerical values of this pressure. Refinement of analysis and method may alter the absolute values of our figures. In any event we must bear in mind that the respiration pressures are a function of depth and time interval of the respiratory act and may, and do, vary greatly *in* the individual as well as *among* individuals, as has been known all along and as our experiments show. The big gap in our knowledge has been *how much* does blood pressure vary with respiration, and *how much can it be made to vary*. Our figures answer these questions more definitely than heretofore has been attempted.

It should be stated here again that during our experiments, while the subjects were asked to breathe deeply and slowly, they were asked to do so only to the extent to which they could still feel comfortable. Had they breathed to the fullest capacity of their lungs the values of respiration-pulse-pressure would doubtless have been still greater than these we have recorded.

In any case our experiments show beyond a doubt that during deep breathing there is an ebb and flow of the blood throughout the tissues on a much greater scale than physiologists probably would have supposed.

When one looks at the unexpected magnitude of respiration-pulse-pressure one is inclined to question the reckless recommendation to the innocent public of deep-breathing as a harmless form of physical exercise.

Just as pulse pressure has been of so much significance to the physiologist, physical director and clinician, it is believed that the determination of respiration-pulse-pressure, when once sufficient data are gathered, may prove to be of even more far reaching service in the estimation of physical fitness, in the determination of the character of and damages done by disease, and possibly in the more intelligent prescription of physical exercise.

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SUMMARY

1. A method for the determination of the character of respiratory change of arterial pressure in man by means of the Korotkoff sounds is described.

2. An extension of this method is further described whereby one may determine the extent of arterial pressure changes in man accompanying respirations. The pressure change so determined may be expressed in terms of mm. of Hg.

3. The difference between the maximum systolic and minimum diastolic pressures occurring during a respiration has been called the *respiration-pulse-pressure.*

4. In deep breathing experiments it is shown that the respiration-pulse-pressure may be from 19 to 58 per cent greater than the ordinary pulse pressure.

5. A discussion of the significance of the Korotkoff sounds and of their relation to certain characteristics in the corresponding sphygmomanometer tracing, and to the critical arterial pressures in man, is included in the body of the paper.

6. It is shown that the extent and duration of the obliteration of the artery in a decompression experiment may be seen in the sphygmomanometer tracing by the flattening of the troughs of the pulse waves.

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THE CONDUCTION OF PAINFUL AFFERENT IMPULSES IN THE SPINAL NERVES

STUDIES IN VASOMOTOR REFLEX ARCS. II[1]

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Within the skin are endorgans capable of responding to tactile, thermal and painful stimuli. Impulses arising in such endorgans are propagated along afferent nerve fibers to the central nervous system. Within the spinal cord the tactile, thermal and painful afferent impulses follow separate paths toward the brain—paths which have been mapped with considerable accuracy. But we have not known whether in a peripheral nerve a single fiber could convey only one, or several kinds of afferent impulses. If it be assumed that a specific set of fibers mediates each variety of cutaneous sensation, how may we differentiate, for example, those mediating pain from the other afferent fibers? This entire question has been very obscure because of the absence of any adequate knowledge as to the structure and function of the afferent fibers in the peripheral nerves. Only recently has our information along these lines become sufficiently precise to make possible a solution of the problem.

We have attacked the problem along lines suggested by these recent observations and have conducted a series of experiments the results of which are very convincing. It will be necessary, before giving an account of the experiments, to summarize the recent anatomical and physiological observations, a consideration of which led us to make the experiments.

[1] The first paper of this series was published under the title "The conduction within the spinal cord of the afferent impulses producing pain and the vasomotor reflexes," this Journal, xxxviii, p. 128.

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STRUCTURE OF THE AFFERENT CEREBROSPINAL NERVE FIBERS

During the last six years (1) it has been shown by means of a new differential axon stain that the spinal nerves contain more unmyelinated than myelinated fibers. These numerous unmyelinated fibers had not been seen before because they could not be stained by any of the methods previously used. They have been demonstrated in great numbers in the vagus as well as in the spinal nerves and are probably present in other cranial nerves also. It has been shown that these fibers in the spinal nerves arise from the small cells of the spinal ganglia. These, like the larger cells of the ganglia, are unipolar with a process that divides dichotomously. One branch runs peripherally along the spinal nerve, the other runs centrally along the dorsal roots to the spinal cord. Both remain unmyelinated throughout their course. That these small cells of the spinal ganglion and the associated unmyelinated fibers are afferent elements is shown by the location of the cell body in the spinal ganglion and its conformity to the typical structural type of afferent neurones.

Most of the unmyelinated fibers in the spinal nerves go to the skin; a few go to the deeper structures. Traced centrally along the dorsal roots, they are seen to run into the tract of Lissauer of the spinal cord. As the root approaches the spinal cord it breaks up into a number of fine radicles which spread out in a longitudinal direction and enter the cord along the posterolateral sulcus. Within each radicle as it approaches this sulcus the unmyelinated separate out from among the myelinated fibers and take up a position around the circumference of the radicle and along septa that divide it into smaller bundles. Then, as indicated in figure 1, these unmyelinated fibers run toward the lateral side of the radicle and, leaving it just as it enters the cord, they turn ventrolaterally into the tract of Lissauer. They are accompanied by a few fine myelinated fibers; but almost all of the myelinated fibers run medialward over the substantia gelatinosa into the fasciculus cuneatus. This fasciculus receives practically none of the unmyelinated fibers. We shall speak of the bundle of unmyelinated fibers that turns ventrolaterally into the tract of Lissauer as the lateral division of the root, and of the myelinated fibers that run over the substantia gelatinosa into the cuneate fasciculus as the medial division. The fibers of the lateral division are extremely fine and closely packed together, so that it is small as compared to the medial division, which consists of very much coarser fibers. Yet in spite of its small size, the lateral

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contains fully as many if not more fibers than the medial division, as can be readily understood when one considers the great difference in the size of the contained fibers. The number of the unmyelinated fibers could not be adequately represented in a low power drawing like that represented in figure 1.

The unmyelinated fibers run up or down in Lissauer's tract for only a very short distance, usually less than a segment. They then turn into and end in the substantia gelatinosa, which is to be regarded as the nucleus of reception of these fibers. That is to say, these fibers run into the gray matter at or near the level at which they enter the cord. Their intraspinal course suggests at once that they are the fibers of pain and temperature sensations, since it is known that the afferent impulses underlying these sensations pass through the gray matter as soon as they reach the cord. This brings us to a consideration of Head's important work on pain and temperature sensations.

PROTOPATHIC NERVE FIBERS

An important advance was made by Head and his associates (2) when they showed that cutaneous sensations could be separated into two groups, to which they applied the terms protopathic and epicritic. Under the term protopathic Head groups pain and the temperature sensations aroused by objects under 22° or over 40°C. This group is characterized by a "peculiar tingling quality," by radiation into other parts than those stimulated, and by failure of the subject to localize accurately the point stimulated. Under the term epicritic he groups sensibility to light touch, temperature sensations derived from objects between 22° and 40°C., and discrimination of the two compass points. Sensations of this sort are all accurately localized. It would take too much space to tell in detail how these two types of sensation were separated from each other. Obviously such a distinction could not be made by a study of the normal skin. It was found, however, that after lesions of the dorsal roots areas of pure epicritic sensation appeared. Such a cutaneous area was sensitive to light touch and to medium degrees of temperature, but insensitive to pain and to the more extreme degrees of temperature. On the other hand, when the median nerve was cut a cutaneous field became outlined on the palm of the hand, in which only protopathic sensations were experienced. Here the skin was sensitive to pain and to the extreme degrees of temperature, but insensitive to light touch and the intermediate degrees of temperature.

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All sensations from such an area were poorly localized and had a peculiar tingling quality. Head and his associates studied a very large series of cases with nerve lesions and found many such areas of dissociated sensation.

It is maintained by Head that each of his two sensory groups depends on a separate anatomically distinct set of nerve fibers. He presents good and convincing reasons for this belief, but space does not permit us to repeat the argument here. We wish only to call attention to a fact which might easily be overlooked in reading the original articles. In areas of partial anaesthesia the residual sensation may be either protopathic or epicritic. If the only form of residual sensation were protopathic one might assume that it depended only on a decreased density of innervation. It might easily be that light touch required a denser innervation for its perception than pain. But the reverse form of partial anaesthesia also occurs (and numerous examples of it are given by Head) in which epicritic sensation persists over an area devoid of protopathic sensation. It is not conceivable that a simple decreased density of innervation should in the one case give rise to a loss of light touch with pain persisting and in another case cause a complete loss of pain sense while light touch remains normal. Furthermore, these areas of pure epicritic sensation are sensitive to temperature between 22° and 40°, but insensitive to the more extreme degrees of temperature. This is clearly not a case of lowered sensibility due to decreased density of innervation. It seems clear to us that these facts can be explained only on Head's assumption that there are two kinds of afferent nerve fibers, which differ slightly in their anatomical distribution.

According to Head, the unit of distribution of protopathic fibers is the dorsal root, each root having a sharply outlined area of skin which it supplies with them. The epicritic fibers of adjacent roots are intermingled in their cutaneous distribution. Section of one or more dorsal roots deprives a sharply circumscribed area of skin of its protopathic fibers, while epicritic fibers from adjacent roots run into this area, endowing more or less of the skin near its border with pure epicritic sensation. Here light touch is felt, but not pain, warm and cold objects are discriminated but hot and cold objects give rise to no temperature sensation. In the same way the peripheral nerve is the unit of epicritic sensation. The epicritic fibers of the ulnar nerve are limited to the area of skin outlined by anatomists as representing the cutaneous distribution of that nerve; but the protopathic fibers of the ulnar run

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long distances in the subcutaneous plexuses into the areas belonging to adjacent nerves. When the median nerve is cut epicritic sensation is lost over the entire area ordinarily assigned by anatomists to that nerve, but protopathic fibers from the ulnar nerve run into this area endowing a considerable extent of the skin near the border of the area with pure protopathic sensation. Here pain is felt but not touch. Hot and cold objects are distinguished, but warm and cool objects give rise to no temperature sensation.

Facts which are otherwise inexplicable are thus readily understood on the assumption of two kinds of nerve fibers which vary slightly in their anatomical distribution. This assumption acquires still greater significance in view of the recent demonstration that there are two kinds of afferent cerebrospinal nerve fibers which differ both in structure and distribution.

As we have seen in the first section of this paper, there are in the cerebrospinal nerves, great numbers of unmyelinated fibers which had been previously overlooked. The most striking parallel exists between what is known of the protopathic fibers and what has recently been determined in regard to the unmyelinated fibers. This comparison can be carried out with great detail and with the most convincing results; but it involves many details which have no place in this paper and have been presented elsewhere (3). The course of the afferent fibers in the dorsal root and spinal cord, has, however, a direct bearing on the present investigation and must be considered here.

It is well known that the afferent impulses underlying sensations of pain and temperature must pass through the gray matter and cross to the opposite side of the cord at or near the level at which they reach it. Head (2) has shown that this is true for temperature sensation of the epicritic as well as of the protopathic order. According to him, the other elements of the epicritic group (touch, tactile discrimination and tactile localization), are carried upward on the same side of the cord in the posterior funiculus for varying distances before ending in the gray matter. He maintains that the tactile impulses coming in along a given root do not cross to the opposite side of the cord all at once, but that they ascend in the posterior columns for varying distances. The crossing at various levels, of impulses coming in by a single root gives rise to a double pathway for touch, uncrossed fibers of the first order paralleling crossed fibers of the second order for a certain number of segments. This double path no doubt accounts for the conflicting observations on the conduction of tactile impulses, which are found in the literature.

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The facts that have been ascertained regarding the intraspinal course of the unmyelinated fibers are in complete accord with the view that they are the conductors of protopathic afferent impulses. As a dorsal root enters the spinal cord the two kinds of fibers separate; the unmyelinated turn laterally into Lissauer's tract, while the myelinated run on into the posterior funiculus. Few, if any, unmyelinated fibers enter that funiculus, but a few fine myelinated fibers run into the tract of Lissauer. This consists chiefly of unmyelinated axons, scattered among which are a few fine myelinated fibers. From the level at which they enter the cord these fibers ascend or descend in the tract for a very short distance not exceeding one or two segments. The substantia gelatinosa seems to be the sensory nucleus associated with this tract.

The unmyelinated fibers, then, enter the gray matter at or near the level at which they enter the cord. In this they are in exact agreement with the fibers conveying protopathic sensation. The myelinated fibers, which alone enter the posterior funiculus, correspond in their intramedullary course to the fibers carrying light touch, tactile discrimination and tactile localization, since according to Head these ascend for longer or shorter distances in this funiculus before entering the gray matter. As to the temperature sensations in the epicritic range, they are probably conveyed by the fine myelinated dorsal root fibers that run with the unmyelinated ones into the tract of Lissauer. It is thus apparent that we have at hand data sufficient to explain the intramedullary course of the protopathic and epicritic sensations in terms of the demonstrated intramedullary course of the myelinated and unmyelinated fibers.

The function of the tract of Lissauer. Experiments on the spinal cord of the cat (4) have shown that the tract of Lissauer and the substantial gelatinosa Rolandi are at least closely associated with the pain reception and conduction apparatus. It was found that while bilateral destruction of the tract of Lissauer and the substantia gelatinosa at the level of the first lumbar segment of the cat's cord did not interfere in any way with the perception of pain in the hind limb, it entirely eliminated the pressor vasomotor reflex from stimulation of the sciatic nerve. Now, the vasomotor reflexes are distinctly protopathic in that they are produced almost exclusively by pain and temperature sensations. The evidence presented in that paper showed that the tract of Lissauer and the substantia gelatinosa formed a path for the conduction of the afferent impulses involved in the reflex vasoconstriction due to painful sciatic stimulation. It seemed probable to us that the tract

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of Lissauer and the substantia gelatinosa Rolandi formed an apparatus for the reception and intersegmental conduction of painful afferent impulses. Some impulses from this apparatus passing over to the spinotholamic tract would reach the cortex and find expression as conscious pain, while other impulses received in this apparatus would ascend and descend within it, producing pain reflexes. So far as the evidence goes, this work favors the theory that the unmyelinated fibers conduct protopathic sensation, in that it shows that the portion of the cord in which these fibers run and terminate forms part of a protopathic reflex arc.

STATEMENT OF THE PROBLEM AND OUTLINE OF THE EXPERIMENT

It occurred to us that the sharp separation of dorsal root fibers to form the medial and lateral divisions of the root could be made the basis of some interesting experiments. By raising the root and cutting in the direction of the arrow *A* in figure 1, the lateral could be cut without injuring the medial division. On the other hand, by a cut in the direction of line *B* in figure 1 the medial could be cut without injuring the lateral division. Stimulation of a root before and after such an operation might yield information concerning the sort of afferent impulses that enter the cord by way of each of these two divisions of the root, and concerning the function of the myelinated and unmyelinated fibers.

TECHNIQUE

Adult cats were used for the experiment. The spinal canal was opened by removal of the spinous processes and laminae from the fifth lumbar to the first sacral vertebra inclusive. In all but the first three experiments this was done as a preliminary operation under rigid asepsis and the animals were allowed to recover from the loss of blood and shock of this operation for a period of five to ten days. The three cats in which the exposure and experiment were carried out under one anaesthetic had a very low blood pressure, but those which were allowed to recover from the preliminary operation showed a normal blood pressure during the subsequent experiment.

During the experiment the animals were under ether anaesthesia. A tracheotomy was performed and an ether bottle attached. Care was taken to maintain a constant and rather light grade of anaesthesia. Connections were made to secure carotid blood pressure tracings. The animal was then placed on an animal board so arranged that the weight

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was borne entirely by the upper part of the thorax and the pelvis—the lower part of the thorax and the abdomen being free to move during respiration without coming in contact with the board.

The dura mater was opened by a median dorsal incision corresponding to the length of the defect in the bony canal. The last large root was selected for the experiment, and at the autopsy this was found

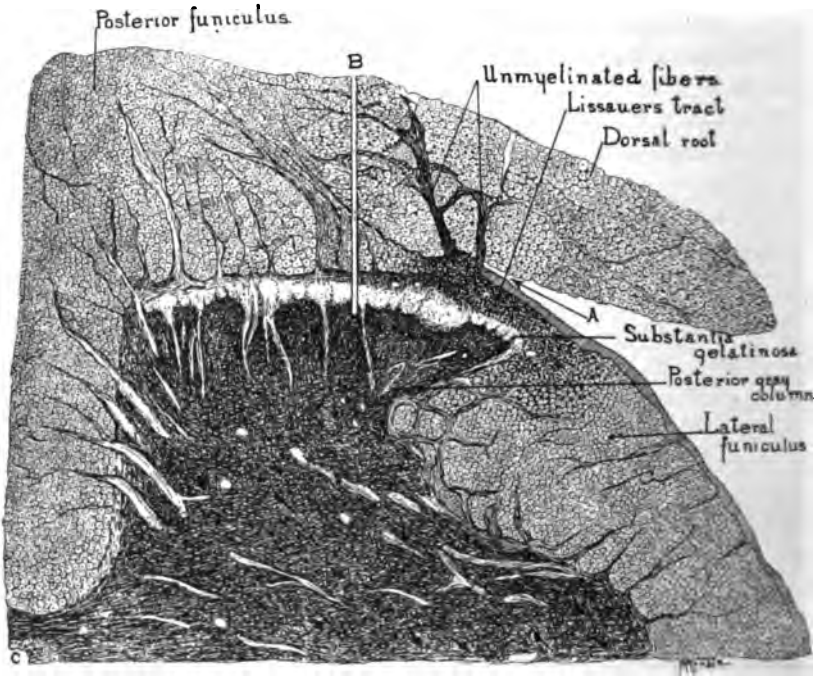


FIG. 1. From a section of the seventh lumbar segment of the spinal cord of the cat. Arrow *A* indicates the direction of a cut through the lateral division of the root. Line *B* indicates the direction and extent of a cut through the medial division of the root.

to have been the last lumbar in every case except two in which it was the first sacral. A ligature was passed around the root selected at the level of the ganglion; the ligature was tied and the nerve cut distal to it. The nerve was raised by traction on the ligating thread and the ventral root was divided just proximal to the ligature. The preparation then consisted essentially of a dorsal root ligated and cut distal to the ligature, but still attached centrally to the spinal cord. The sev-

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enth lumbar and first sacral roots are long and furnished a preparation 2 or 3 cm. in length. They were chosen partly because of their length and partly because the separation into medial and lateral divisions is very evident in these roots. The two divisions are so placed as to be readily divided separately.

During stimulation the root was elevated by gentle traction on the ligating thread. Standard platinum electrodes were applied a short distance proximal to the ligature. The stimulus was a faradic current derived from a Stoelting inductorium No. 7090, through the primary of which was passed a constant half ampere current. The position of the secondary varied from 5 to 8. Note was made of the changes in respiration and any struggling produced by the stimulus and the vasomotor reflexes were recorded on the kymograph.

After a record had been taken of the results of stimulating the root, the desired cut was made with a sharp iridectomy knife. The knife was drawn carefully along either the medial or the lateral side of the entering root. The incisions along the lateral side of the root in the direction of the arrow in figure 1 were always very restricted in extent. The cuts on the medial side varied, in two cases extending deeply into the cord as indicated by the line *B* in figure 1. After the cut had been made the root was stimulated again in the same manner and with the same strength of current as before.

The rootlets into which each root divides as it enters the cord are very small and the preparation requires careful handling. On account of the small size of the rootlets it was felt that there was danger that they might become chilled or dry during the experiment. In order to prevent such an error the cord and roots were kept flooded with normal salt solution at 39°C., except during the time of stimulation. Preceding each stimulation the saline was removed with absorbent cotton and the cut end of the root elevated by gentle traction on the ligating thread. Since the preparation was at least 2 cm. long and the stimulus was applied close to the ligature, there was no danger of an escape of current to the cord or other roots.

After one root had been tested in this way the corresponding root of the opposite side was used. In most of the cats the medial division of one root was cut and the lateral division of the other. In this way it was possible to compare the effects of the two lesions in the same cat under the same conditions of anaesthesia, blood pressure and vasomotor irritability.

The animal was then autopsied and the roots identified. They were

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usually the seventh lumbar, but twice the first sacral. The roots and corresponding segment of the cord were removed together and cut into serial sections stained by the pyridine silver technique. These sections were studied under the microscope and the exact amount and character of the damage done in each case was determined.

SECTION OF THE LATERAL DIVISION OF THE DORSAL ROOT

The lateral division of the root was cut in three first sacral nerves—on one side in one cat and on both sides in another cat—and in three seventh lumbar nerves—one nerve in each of three cats. In two of these six experiments stimulation of the root preceding the section of the lateral division gave rise to some struggling, although the animals were under moderately deep anaesthesia. This struggling was not elicited by stimulation of the same root with the same strength of current after the lateral division had been cut, although it could still be elicited by stimulating other nerve roots. Stimulation of the intact root resulted in an increase in rate and depth of respiration; the same stimulation after section of the lateral division gave rise to no change in respiration. In each of the six experiments stimulation of the intact root caused an increase in blood pressure—the typical pressor curve—and in each instance the pressor reflex was found to have disappeared after section of the lateral division of the root. In most cases these results were checked by a subsequent stimulation of another root which gave both the pressor reflex and changes in respiration showing that the respiratory and vasomotor centers were still functioning normally. It is evident that the lesion, which was a very superficial one on the lateral aspect of the entering root, prevented the entrance into the cord of those afferent impulses that cause struggling, increased rate and depth of respiration, and the pressor vasomotor reflex. In four experiments the pressor reflex was entirely obliterated and in the other two there remained only the slightest trace of this reflex.

In each case the cord segment and dorsal root involved were stained by the pyridine silver method and cut into serial sections which were carefully studied under the microscope. These showed that the lesion had been accurately placed and was very restricted. Although a majority of the fibers of the lateral division had been cut, some had escaped. In only one experiment was the division of this part of the root complete; but in every case it had suffered serious damage. In none of these experiments had the medial division of the root been injured.

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The medullated fibers of the root could be followed into the cuneate fasciculus and showed no evidence of having been damaged by the laterally placed cut.

As an illustrarion of the results obtained we will cite the details of one experiment:

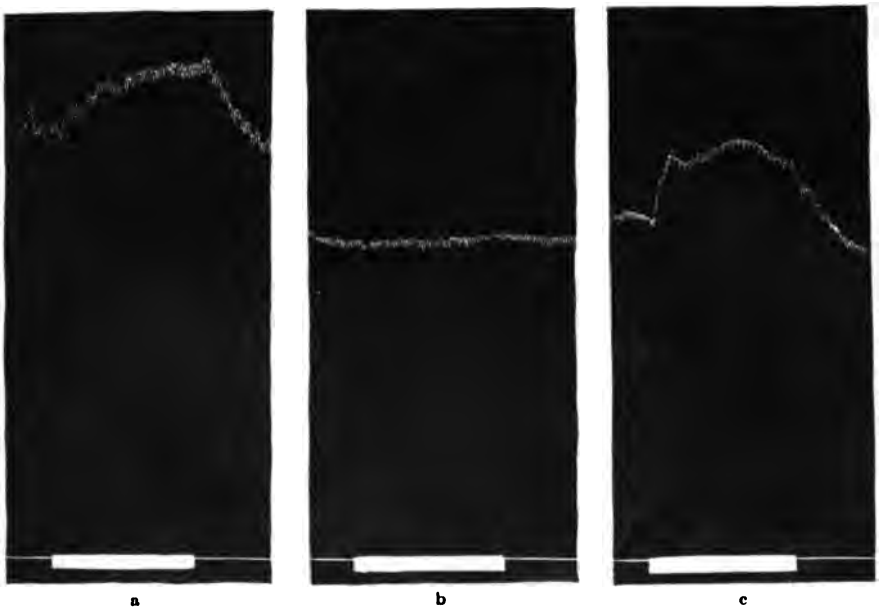


FIG. 2. Carotid blood pressure tracing from Cat 64. *a*, Strong faradic stimulation of the left seventh lumbar dorsal root; *b*, same stimulation of the root after its lateral division had been severed; *c*, same stimulation of the right seventh lumbar root after a cut had been made on its medial side as extensive as the cut on the lateral side of the left root.

Cat. 64, adult. Preliminary operation of opening the spinal canal December 3, 1915. Experiment December 14, 1915. Ether anaesthesia. Wound in skin and muscles opened and dura exposed. Tracheotomy. Ether bottle. Carotid canula. Dura opened. Cord and roots kept flooded with warm normal salt solution except during stimulation. Ligature passed around the left seventh lumbar nerve, tied, and the nerve cut distally. Ventral root cut near the ligature. Dorsal root gently raised by the ligating thread and electrodes applied close to the ligature. Faradic stimulation fifteen seconds with the secondary coil at 5. Result: Good pressor reflex—figure 2a; increased rate and depth of respiration, the increased rate being indicated in figure 2a by the obliteration of the respiratory wave in the blood pressure tracing; some struggling. A very small cut along the lateral side of the root in the direction of the arrow in figure 1. Root

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stimulated as before. Result: No struggling; no change in respiration; no change in blood pressure—figure 2b. A study of serial sections shows that the lateral division of the root was completely cut with practically no injury to the medial division.

SECTION OF THE MEDIAL DIVISION OF THE DORSAL ROOT

To the preceding experiments the objections might well be raised that the rootlets, emerging in linear order from the posterolateral sulcus and uniting to form the dorsal root, are so very fine that it would be impossible to make the cut described without traumatizing them to such an extent as to render all the fibers nonconductive. In order to meet this objection it was necessary only to subject the medial side of the root to the same amount of trauma. Now, the medial division is much larger than the lateral and a cut which was sufficient to sever the lateral would be only a superficial cut if applied to the medial division. If the results of the preceding series of experiments had been due to trauma to the root as a whole the same results should be obtained when that trauma involved the medial instead of the lateral side. One such experiment was performed. In Cat 64 the right seventh lumbar dorsal root was prepared for the experiment and stimulated as in the preceding experiments. Struggling, changes in respiration and a pressor vasomotor reflex resulted. Then a cut was made along the medial side of the entering root fully equal to that made in five out of six of the experiments on the lateral division. This cut, which must have traumatized the root as a whole as much as did the lateral cuts, was nevertheless without effect on the pain reflexes. Stimulation of the damaged root brought out the struggling, changes in respiration and pressor reflex just as it did before the lesion, and these pain reflexes were undiminished—figure 2c. It is evident, therefore, that the injury to the lateral side of the root had the effect of eliminating the pain reflexes not because of general trauma to all the fibers of the root, but because of the specific lesion in the lateral division of the root which involved the unmyelinated fibers.

In two other experiments the cut on the medial side was made more boldly and extended into the cord along the line *B* in figure 1, cutting off the medial division of the root as it runs obliquely upward into the fasciculus cuneatus. In one of these, Cat 61, a study of serial sections shows that the medial division of each and every radicle of the left seventh lumbar root was completely severed along the line of *B* in figure 1. In the other, Cat 66, the medial division of the highest radicles of

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the root were incompletely severed and a considerable number of medullated fibers from these radicles ran cephalad on the lateral side of the cut to reach the fasciculus cuneatus. It is true that a few medullated fibers make their way into the posterior gray column lateral to the line of the incision. But it may be conservatively estimated that in Cat 66 more than 75 per cent of the medullated fibers of the root were cut, and in Cat 61 more than 90 per cent, and yet in neither of these experiments were the pain reflexes abolished. Figure 3 represents a tracing from Cat 61 and shows that after section of the majority of its medullated fibers (estimated at 90 per cent) stimulation of the seventh lumbar root gave a good pressor reflex. It is true that the rise was 30 per cent less than that produced by the same stimulation before the medial cut was made, but this was to be expected. It is remarkable that so extensive a cut could be made so close to the lateral division of the root without traumatizing that part of the root more extensively than is indicated by a 30 per cent decrease in its conductivity. In Cat 66 the pain reflexes were not at all decreased by section of the medial division of the root. This experiment shows conclusively that after the great majority of the myelinated fibers have been cut stimulation of the root still gives a good pressor reflex. This is very significant in connection with the fact that division of the majority of the unmyelinated fibers through a relatively small lesion on the later side of the root completely abolished the pressor reflex. In Cat 66, after section of the medial division of the root stimulation still caused an increase in depth of respiration and doubled the rate. We have no record of the respirations in Cat 61.



FIG. 3. Carotid blood pressure tracing Cat 61. Strong faradic stimulation of the left seventh lumbar dorsal root after the medial division had been completely severed along the line *B* in figure 1.

From the results of these last two experiments it may be concluded that the afferent impulses which cause changes in respiration and the pressor vasomotor reflex are not conveyed by the medial division of the dorsal roots.

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INTERPRETATION OF RESULTS

We have shown that the afferent impulses producing struggling, increased rate and depth of respiration and the pressor vasomoror reflex are conducted along the lateral division of the dorsal root and not along the medial division. Now, practically, all of the fibers in the medial division are myelinated and the great majority of those in the lateral division are unmyelinated. Our results may, therefore, be restated as follows: The reflexes mentioned are abolished whenever most of the unmyelinated fibers are cut, but remain unaffected when a majority of the myelinated fibers have been divided. The conclusion cannot be avoided that the afferent impulses bringing about these reflexes are mediated by unmyelinated fibers.

Struggling and the changes in respiration and blood pressure which have been described have always been regarded as reflexes produced by painful afferent impulses and we believe that we may safely conclude from our experiments that the afferent impulses underlying conscious pain also reach the cord by way of the unmyelinated fibers, although in the nature of things this can never be absolutely proven by animal experiments.

Pain belongs to Head's group of protopathic sensations. He believes that the sensations of this group—pain and the temperature sensations aroused by objects under 22° or over 40°C.—are mediated by a special set of nerve fibers. As has been seen in a preceding paragraph, the most striking parallel exists between what is known of the protopathic fibers and what we have learned concerning the unmyelinated fibers. In this paper we have presented evidence that at least the pain element of protopathic sensibility is conveyed by these fibers. On the basis of the rapidly accumulating evidence, we believe that the unmyelinated fibers mediate not only pain, but the protopathic temperature sensations as well.

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IV. DIFFERENCES IN RHYTHMICITY AND TONE IN DIFFERENT PARTS OF THE WALL OF THE STOMACH

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Before taking up the differences observed in the stomach, it may be of interest to review briefly the theoretical considerations that led up to the work. When it was seen that the rate of rhythmic contracton in the small intestine varies inversely as the distance from the pylorus (1), the next question to arise was: If this part of the primitive intestinal tube behaves in this way, how about the other parts? Might not the tract have been constructed originally so that the rate would be highest at the pharynx and lowest at the anus? Although this question cannot be answered satisfactorily as yet, there is considerable evidence in favor of such a view. For instance, the rates of contraction in different parts of the colon (of the rabbit and cat) fit quite well into a prolongation of the curve plotted from the rates of the small bowel (2). The rhythm varies (in the rabbit) from 6 to 10 per minute near the cecum to from 3 to 5 per minute near the anus. It is impossible to say much about the esophagus of mammals because, in them, that tube is made up almost entirely of striated muscle, and the smooth fibers, with which we are concerned, appear only in the lower third or fourth. Longitudinal segments from this region near the cardia (in rabbits and cats) showed a high rhythmicity when placed in aerated Ringer's solution. The fastest rate seen in the cat was 14 per minute; in the rabbit it was sometimes as high as 19 per minute. It should be noted that this is a higher rate than that ever seen in the duodenal segments (15 to 17.5 per minute).

We can compare the rhythm of different regions of the esophagus only in those lower animals in which the tube is made up entirely of smooth muscle. This is the case in the frog. Stiles found in the esophagus of this animal that the rhythmic activity is more marked and

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regular than in any other part of the digestive tract; also, that the rate of contraction (in the esophagus) varies inversely as the distance from the pharyngeal end (3). I have confirmed these findings in a number of frogs; and, although my tracings are not so regular as Stiles', they show the difference in rate very clearly. I have found similar differences in longitudinal segments from the esophagus of a small grass snake (species unknown). The contractions in the intestinal segments from the frog unfortunately were so irregular that I could not establish a further gradation of rhythm from the end of the esophagus down to the cloaca. The only thing that can be said is that the esophageal rates were generally faster than those of any part of the bowel.

Even if further work on such animals should show definitely a gradation of the rhythmic activity from pharynx to anus, we would still have to explain the slow rhythm of the gastric waves in mammals: from 3 to 4 per minute in the rabbit, dog and man, and from 4 to 6 per minute in the cat. A possible way out of this difficulty was suggested to me by the literature on another muscular tube—the heart. Gaskell taught us to view that organ as an elaboration of a simple tube which had become twisted on itself, and had bulged in places. There the muscle became specialized that it might contract and empty the cavities more quickly. "The development of this nearer approach to striated muscle is made at the expense of the original rhythmical power" (4).

THE PRIMITIVE TUBE

A glance at figures 1 to 34 in Oppel's Comparative Histology (5), or at plates 18 to 33 in Huntington's book (6), will show how the stomach also has been evolved from a simple tube, first by an enlargement, secondly, by a bending of the pylorus towards the cardia, and thirdly, by the addition of cecal pouches. The stomach of the eel consists almost entirely of such a pouch, which has grown from the convex side of a bend in the original tube (see fig. 1, *D*). It is very obvious, in such a stomach, that the primitive tube is to be found along the lesser curvature. Even the complicated stomachs of ruminants can be resolved into a series of ceca arranged along the orginal tube (fig. 1, *E*). That part of the fundus to the left of the cardia in the human stomach represents such a cecum, which, very early in life, grows out from the greater curvature (7). The stomach of a 10 mm. human embryo is made up of three parts: the expanded, conical, lower end of the esophagus, the long tubular antrum, little larger than the adjacent duodenum, and a

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small fundus (fig. 1. *F*). The end of the esophagus meets the antrum at the incisura angularis. Later, the fundus grows at the expense of the other two parts, so that, in the adult, the end of the esophagus is represented only by the cardiac antrum and that prolongation along the lesser curvature which forms the gastric canal; while the pyloric antrum makes up a much smaller part of the stomach than it did originally (8).

The "Primitive Tube," accordingly, must be looked for along the lesser curvature. It is suggestive that this part of the stomach is

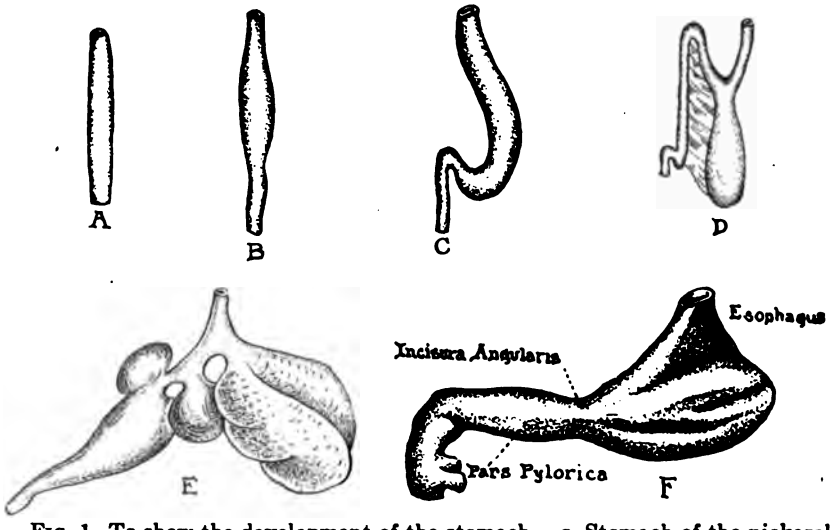


FIG. 1. To show the development of the stomach. *a*, Stomach of the pickerel (Nuhn); *b*, stomach of Proteus anguineus (Nuhn); *c*, stomach of Scincus ocellatus (Nuhn); *d*, stomach of the eel (Huntington); *e*, scheme of the ruminant compound stomach (Nuhn); *f*, stomach of a 10 mm. human embryo (Lewis).

lined by an epithelium differentiated least of all from that of the intestine. This point has been remarked upon by several men in discussing the mucous membrane of the pyloric antrum. The glands around the cardia are apparently little more than sluggish pyloric glands (9); and "it is almost the rule for the greater part of the mucous membrane along the lesser curvature to be of the pyloric type" (10). A similar arrangement is found in most of the domestic animals, that is, the lesser curvature is lined only by cardiac and pyloric glands (11).

To be sure, we must be careful in comparing conditions in two organs so different in function as are the heart and stomach. One has been

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specialized to pump blood rapidly; the other serves largely as a reservoir, a hopper for the bowel, where the waves do more mixing than propelling. Yet they have both been evolved from simple tubes of rhythmic muscle, and it seems to me that the analogies are close enough to make us eager to examine the stomach from a point of view which has done so much to advance our knowledge of the physiology and pathology of the heart. In the stomach, we should expect to find the most rhythmic tissue at the cardia and along the lesser curvature. The least rhythmic tissue might be in the fundus and along the greater curvature. Such differences, if present, might go far to explain the origin and peculiarities of gastric peristalsis. I wish to show now to what a considerable extent these expectations have been fulfilled.

TECHNIC

After some experimenting, good records were obtained from longitudinal strips of muscle from different parts of the stomach of the rabbit, cat, dog, and man. With a razor, parallel cuts 3 to 5 mm. apart were made just to the mucosa. A narrow strip of muscle 2 cm. long was then lifted up, after cutting through the submucosa with a fine pair of scissors. The only place in the rabbit where this was impossible was along the canalis gastricus from the cardia to the incisura angularis. Here I could find no line of separation, so the muscular strips from this part of the lesser curvature were studied with mucous membrane attached (fig. 2, *A* and *B*). A separation could be made in the cat, dog and man, although it was more difficult in this region than in the rest of the stomach. This close attachment of the mucosa to the muscle brings to mind a similar arrangement of skin and fascia in the palm of the hand, which enables us to grasp things firmly. In the same way, in the stomach, this intimate relation between muscle and mucous membrane may be essential to the formation of the canalis gastricus, through which fluids flow along the lesser curvature (12). A localized contraction on the greater curvature might not show at all on the inside of the stomach, as the mucosa there is redundant, and but loosely attached to the muscle.

Separation of the strips was very easy in the pyloric antrum, and the laxity of the submucosa in that region was striking in all the animals studied.

The strips were usually immersed in warm aerated Ringer's solution and studied at once, but they can be kept in the icebox for four or five

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days. It is remarkable that daily tracings from the same set of strips showed that they might beat even better on the second or third day than on the first. This seemed to be due to a loss of inhibition, as the strips generally began beating more promptly after immersion; the rhythm often was faster, and the records more regular. A great deal of patience was needed with fresh strips, as many of them did not show activity until they had been in the warm Ringer's solution for an hour or more. Even then, one part of barium chloride to 25,000 of the solution often had to be added before some of them would beat. The temperature of the Ringer's solution was kept between 37° and 38°C. Ordinarily no weight was added to the light heart levers used; it was not found to be necessary.

EXPERIMENTAL DATA

The following conclusions are based upon records from the stomachs of sixteen rabbits, eight cats, nine dogs and one man. This material seemed to be sufficient, as most of the data were in such entire agreement. Longitudinal strips from the two curvatures have been used almost exclusively. Some work was done with pieces cut longitudinally midway between the two curvatures and with circular strips from different regions, but it was soon discontinued, as the only ones that showed much activity were those from the neighborhood of the cardia. The type of contraction obtained in the circular strips corresponded to that of the longitudinal ones from the same region.

The first strips to begin contracting after immersion were those from the upper end of the stomach. In the cat and dog the strip from the lesser curvature next to the cardia (fig. 3, *A*) was first, often showing activity immediately after immersion in the bath. In the rabbit, the strips from the fundus (fig. 2, *D* and *E*) seemed to recover sooner from the trauma of attachment and generally became active shortly before the cardiac strips did. Strips from the greater curvature and from the antrum (particularly in the rabbit) often took an hour or two to get started, and even then some did not contract well. Thus, out of eleven strips from the rabbit's antrum, only two showed rhythmic activity.

It should be emphasized that the only strip that could be counted upon in every stomach to give regular, typical tracings was that cut from the lesser curvature near the cardia (*A*, figs. 2 and 3). *This region showed the greatest tendency to rhythmic contraction of any part of the stomach.*

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THE DIFFERENT TYPES OF CURVES

The curves traced by strips from certain regions of the stomach were so characteristic that there was little need for labelling some of them. This might be said particularly about the records of the strip from the lesser curvature of the rabbit near the cardia. The individual

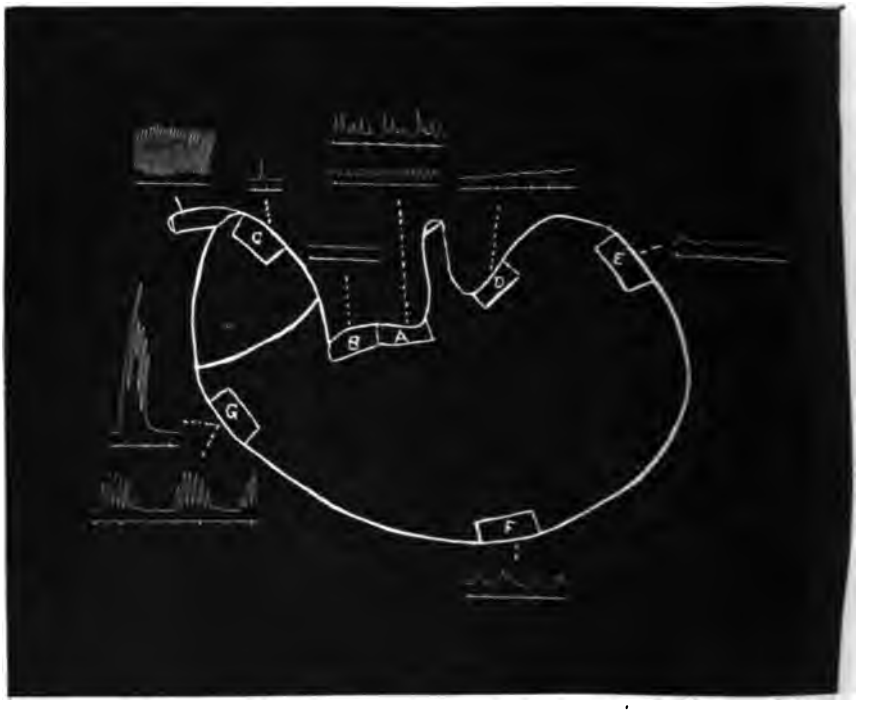


FIG. 2. Diagram of the rabbit's stomach showing the location of the principal strips studied, together with specimen tracings from the different regions. At *A* and *G* two characteristic types of tracing are shown. The time tracing represents 30 second intervals. A short strip of duodenal tracing is inserted for comparison.

contractions of this strip could generally be recognized by the almost vertical rise and the sharp peak caused by the immediate relaxation (see figs. 2 and 4). This type of curve often shaded into another very regular form, particularly when the rate became faster, or after the addition of 1:25,000 $BaCl_2$ (fig. 4). In the cat and dog, the curves from this region could be recognized not only by the sharper apices to the

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contractions and the more rapid rate, but often on account of the peculiar tonus waves depicted in figure 5.

Strips from the lesser curvature (*B*, figs. 2 and 3) beat with a very small amplitude in all the animals. In the rabbit, the waves could sometimes be made out only by using a hand lens. Many strips did not beat at all. The possible reasons for this will be taken up later.

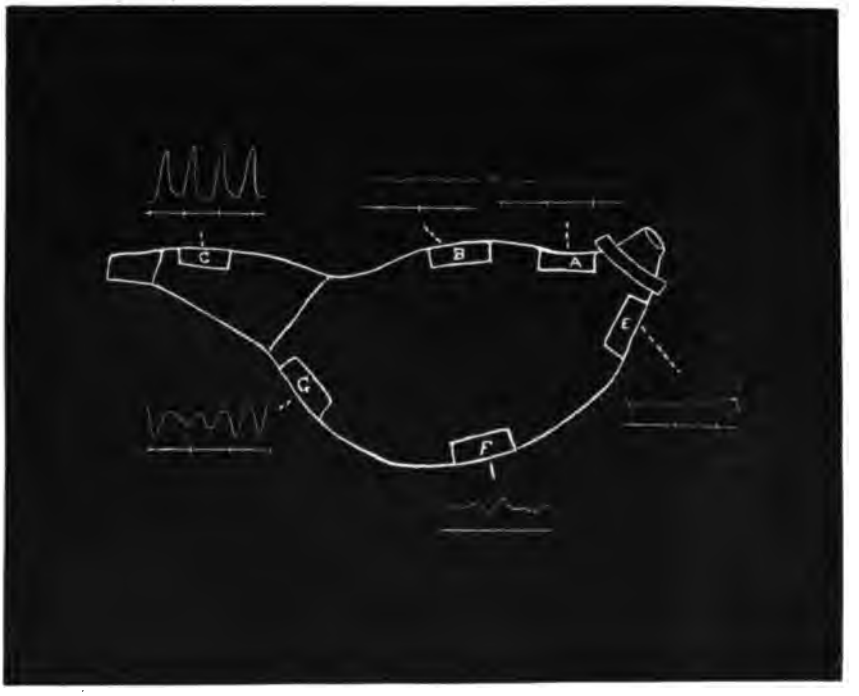


FIG. 3. A diagram of the cat's stomach to show the location of the principal strips studied and the types of tracing peculiar to the different regions. This diagram will serve also for the location of strips in the dog's stomach. The time tracing repres nts 30 second intervals.

In the rabbit, strips next to the cardia on the side towards the fundus (fig. 2, *D*) gave tracings very different from those just described for strip A on the side of the lesser curvature. They were characterized by marked irregularities of tone, rhythm and amplitude. Sections from the rest of the fundus behaved in much the same way. In the cat and dog there was less difference in the behavior of the strips on the two curvatures next to the cardia (fig. 3, *A* and *E*). This is to

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be expected when we remember that their stomachs have almost no fundus and that they are simpler and less differentiated from the original tube.

Strips from the middle region of the greater curvature in all the animals (*F*, figs. 2 and 3) varied a good deal in their reactions. Some did not beat at all, others gave fair tracings, while a few were quite regular. The best curves in the rabbit were seen after the tone had been raised by barium chloride; and particularly in strips which had been on ice from 24 to 48 hours. They never resembled the typical ones from the cardia however. Ordinarily, the waves were large, rounded and uneven. They were even larger and more rounded in the cat and dog.

Strips from the greater curvature near the antrum in the rabbit's stomach gave peculiar curves characterized by regularly recurring groups of waves with a wide amplitude. Sometimes the muscle strip would shorten to less than half its original length. Two such curves are shown at *G*, figure 2. Particularly after the addition of a little barium to the solution, this type of curve often shaded into another, as regular and even as a duodenal tracing. This very regular curve with rapid rhythm was seen in some strips twenty-four hours old from the same region of the cat's stomach. Ordinarily in the cat and dog these strips reacted very much like those from the middle of the greater curvature.

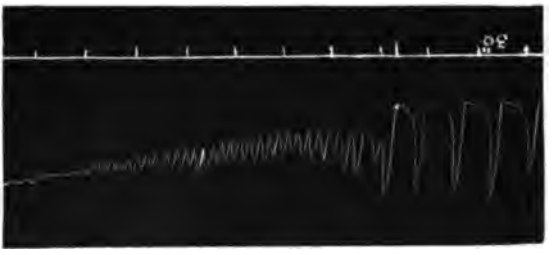


FIG. 4. Tracing from a strip from the lesser curvature near the cardia showing the change in rhythm after adding 1: 25,000 $BaCl_2$.

The type of contraction in the strips from the antrum pylori (*C*, figs. 2 and 3) was very characteristic in all the animals studied. It made no difference from what part of the antrum they were taken. The curves showed a very even base line, upon which were superimposed at regular intervals high symmetrical peaks. These are well shown in figure 6, the middle record. It was very typical even in the frog, where the great amplitude of contraction in this region was well brought out. On such tracings the antral peaks were often four times as high as the cardiac ones, in spite of the fact that the antral strip might

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be only a fourth as long as the strip from the much wider cardiac end of the stomach. This difference is well illustrated by fig. 10 in an article by Woodworth (13)

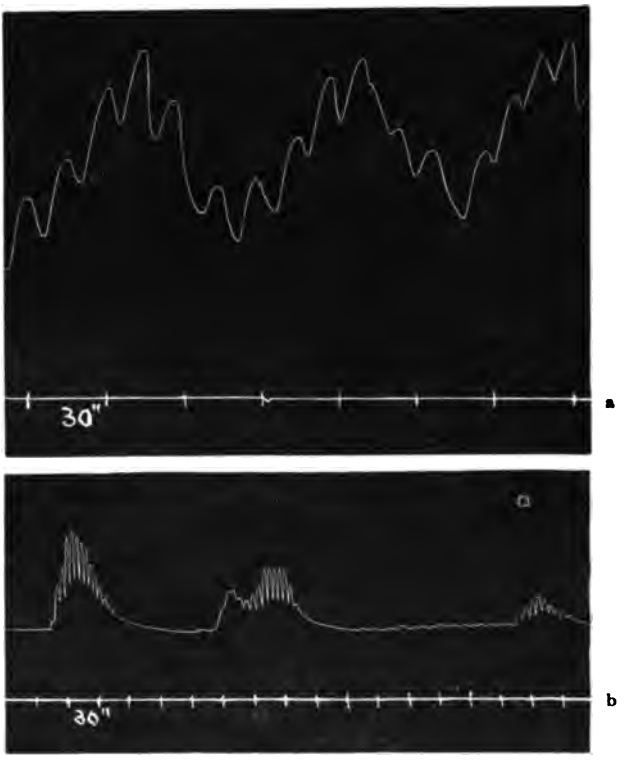


FIG. 5. *a*, Tonus waves in a record from the strip on the lesser curvature next to the cardia of a cat's stomach. *b*, From the same region in a dog's stomach.

DIFFERENCES IN THE RATE OF CONTRACTIONS

Speaking roughly, the rate varied as the distance from the cardia. The fastest rates in all the animals were observed in the tracings from strip A on the lesser curvature. In the cat, this strip contracted from 4 to 8 times per minute. In the rabbit and dog there were two or three different types of curve with different rates. Often when the strips first began to beat after immersion, the rate would be from 2 to 5 per minute. After awhile the contractions would become smaller and more

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frequent, and the rate would change to from 9 to 14 per minute, usually about 11. Three strips beat 20 times per minute for short intervals. The rate in the dog was usually from 8 to 13 per minute, but in a few animals it was from 4 to 7 per minute. At the other end of the stomach, in the antrum, the rates varied from 1 to 4 per minute in all the animals. The rates of the other strips ranged between these two extremes: usually from 4 to 9 per minute. One exception must be made in regard to the strip on the greater curvature next to the antrum in the rabbit(*G*, fig. 2). Here the rate was often from 9 to 12 per minute. The possible significance of this will be discussed later.

DIFFERENCES IN TONE

Differences in tone were observed while studying the strips. When the cuts were made through the muscle on the lesser curvature, the edges pulled apart farther than they did on the greater curvature. Strip A on the lesser curvature was always much smaller than the hole from which it was removed, but strips E and F on the greater curvature might be even larger than the hole, if care were not taken to avoid all traction. Strips E and F would often lie flat when put into Ringer's solution, but strips A, B and G generally curled up tightly.

A high tone on the lesser curvature might have something to do with the poor amplitude of contraction in the strips from this region. I have commented elsewhere (14) upon the fact, so often observed with smooth muscle, that as the tone rises, the amplitude of contraction falls until rhythmic activity may cease entirely. The larger amplitude of contraction seen in the strips from the greater curvature agrees perfectly with the supposition that the tone is low in that region. The great amplitude of contraction in the strips from the antrum and preantrum is probably due to other factors, as the tone seemed to be high. The fibers of the muscle might be longer or more nearly parallel in this region. Such histological differences have been found to explain differences in the reactions of the frog's sartorius and gastrocnemius (15). It is suggestive that McGill (16) has noticed histologically a tendency to almost total contraction of muscle fibers in the pyloric ring.

Tone, unfortunately, is a vague and often misused term. Sherrington has shown recently (17), that many of the phenomena attributed to it are really what he calls "Postural" changes, that is, there is an adjustment of the contractile length of the muscles without necessarily

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an alteration of tension. Such adjustments must take place constantly in the fundus of the stomach so that it can maintain a steady even pressure on the material that is being fed into the rhythmically contracting pyloric mill. This may explain the marked tendency of excised strips from the rabbit's fundus to contract down to about one-half of their original lengths after they have been in the warm Ringer's solution for from 30 to 60 minutes. After this, they seldom relaxed or showed much rhythmic activity. It is interesting to note the resemblance of the tonus waves in a strip from the neighborhood of the dog's cardia (fig. 5b) to those observed by Fano, Porter (18) and others in the auricles of the toad and terrapin. Such changes have been noticed near the cardia in the intact stomach also (19).



FIG. 6. Records from five strips from different parts of the dog's stomach. Shows typical contractions from the pyloric antrum.

THE HUMAN STOMACH

The kindness of Doctors Baxter and Brill enabled me to get the stomach of a man within a half hour after death from nephritis. Strips from this stomach reacted very much like those already studied. Figure 7a shows the small, regular and rapid rhythm in the cardiac strip from the lesser curvature. The rate varied between 5 and 12 per minute. The next piece on the lesser curvature showed a few contractions, only after barium was added. The strips from the preantrum on the lesser curvature contracted very much like those from the greater curvature in some

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cats. The amplitude was large, the rhythm slow and irregular. The strip from the antrum pylori on the greater curvature showed the usual type of curve for that region. Strips from the greater curvature showed less rhythmicity than did those from the lesser curvature.

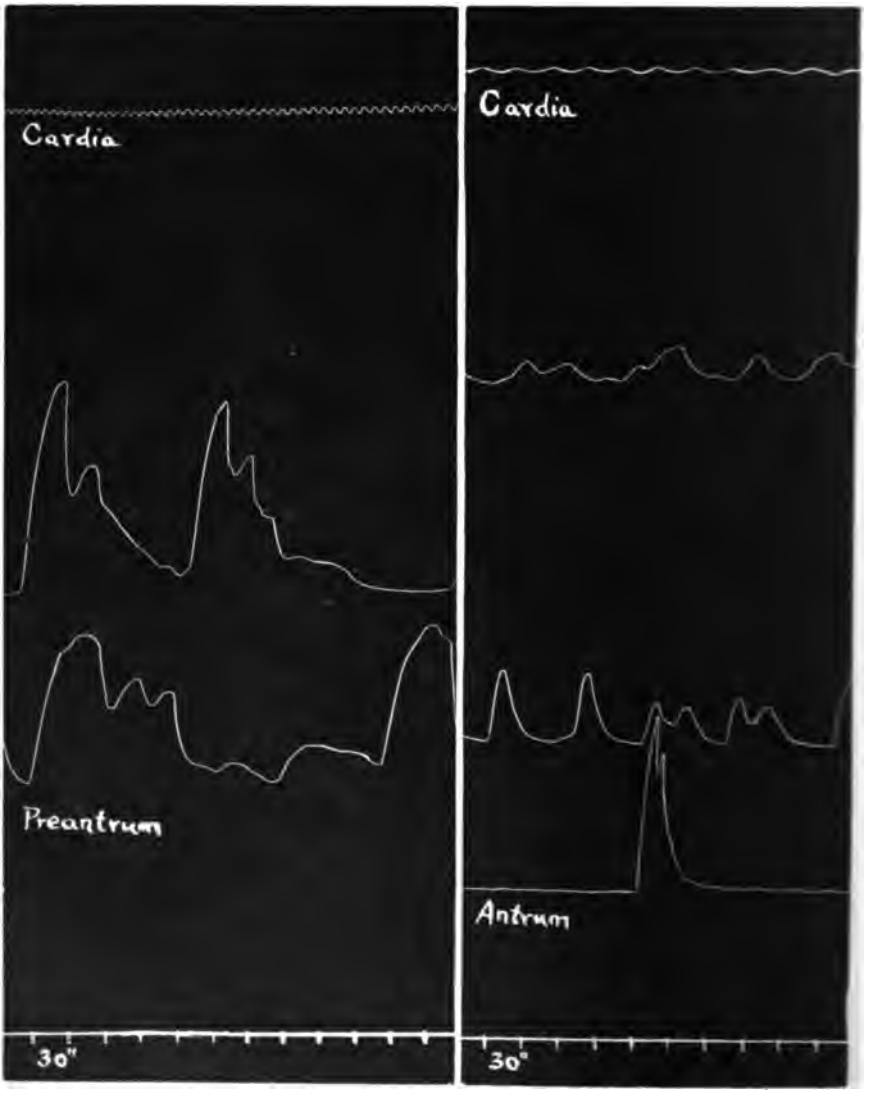


FIG. 7. *a*, Records from four strips from different parts of the lesser curvature of a human stomach. *b* Four strips from the greater curvature.

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DISCUSSION

As was expected, marked differences have been found in the behavior and in the rhythmicity of the strips. Some of these peculiarities, such as the high rhythmicity of strip A, the grading of the rhythm downwards towards the pylorus, and the differences in tone on the two curvatures are explainable on the basis of the theory that gave rise to the work. Other features, such as the low rhythmicity of strips from the middle of the lesser curvature, the promptness with which strips from the rabbit's fundus began contracting after immersion, and the high rhythmicity of the preantral strip on the greater curvature are rather against the view that the rhythmicity should vary inversely as the distance, spacially or embryologically, from the primitive tube.

There are probably other modifying factors present, such as those tending to fit the muscle in the different regions to the different types of work that have to be done. Such a factor might account for the marked differences between the behavior of strips from the pyloric antrum and from the body of the stomach. A remnant of the original tube itself might lose much of its rhythmicity if that function should interfere with the work to be done. This might be one explanation for the poor records obtained from strips from the canalis gastricus along the lesser curvature of the rabbit's stomach. The high rhythmicity of strip G on the greater curvature in the rabbit is not so easily explained. Auer noticed on the intact stomach that after reflex inhibition of the movements, they always returned first in the preantral ring, and he concluded that this was clearly the most rhythmic section (20). I believe, however, that it is exceeded in this regard by the cardia. These problems cannot be settled on the basis of differences in rhythmicity alone. More light must be obtained by studying the irritability, latent period, conductivity, etc., in the different regions. For instance, as will be seen in the next paper, a comparison of the latent periods in different parts of the stomach seems to support the original theory even more than has the study of differences in rhythmicity.

It took many experiments and years of discussion to establish the fact that the stomach can perform its functions quite satisfactorily and normally after section of all extrinsic nerves (21). The observations presented in this paper show now that local peculiarities of tone and rhythmicity may have much to do with directing and modifying the peristaltic wave as it travels over the stomach. A glance at one of

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Groedel's (22) illustrations made up of the superimposed outlines of a dozen serial radiographs of the same human stomach will show how little the lesser curvature, as far as the incisura angularis, is affected by the peristaltic wave. Appearing at a variable distance from the fundus, the waves seem to travel almost entirely along the greater curvature, getting deeper as they approach the antrum. At that point, their character changes markedly; they involve the whole circumference of the stomach and are so deep that they sometimes meet in the center.

It seems to me that these local differences in the peristaltic wave correspond perfectly to the regional peculiarities of tone, rhythm and amplitude of the tissue through which it must pass. As in the heart, so here, the waves probably have their origin in the most highly rhythmic area. Similarly, again, the gradation of rhythmicity from cardia to pylorus may have much to do with maintaining the downward course of the waves.

Conduction must be very different in the two organs, as, in the stomach, the waves keep traveling quite normally after several encircling cuts have been made down to the mucosa (23). Moreover, the pyloric portion of the stomach in dogs continues to functionate normally even after complete separation from the rest of the organ (24). There is also little interference with peristalsis in the human stomach after excising the middle portion for carcinoma (25).

Rather against the view that the waves originate near the cardia is the common observation that they seem to appear now here, now there on the greater curvature. As Cannon says, "the pulsatile source of the gastric waves has no fixed seat" (26). His work showed that a wave is likely to appear at the spot where a certain balance is struck between the tone of the muscle and the internal tension. My records from the intact intestine showed clearly that a peristaltic rush which apparently had begun in the lower ileum had really come as an unnoticed ripple, all the way from the duodenum (27). I believe that the same thing may take place in the stomach, and that ripples sent out from the cardia may deepen into large waves at the place where the conditions defined by Doctor Cannon are right. Perhaps we could see these ripples if we had better means of detecting what is going on. As Groedel (28) says, anyone watching peristalsis in the human stomach would say that the lesser curvature did not participate at all, yet good serial plates always showed waves corresponding to those on the greater curvature. Dietlen (29) has shown also that with the patient lying

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down, so that the fundus is filled, small but definite waves can be seen near the cardia.

Another question that arises is, why should the rates of the strips in the rabbit and dog be so much higher than that of the intact stomach. Only in the cat do they correspond at all. It is different in the intestine, where the rates of the intact bowel and of the excised segments agree quite closely. In the rabbit it may be that the slower, more powerful contractions that were seen in many of the tracings from the cardiac strip are the ones that initiate the peristaltic waves of the stomach. The faster rates may indicate a reserve, of which the cardia has the greatest amount. It does not seem likely that the normal slow rate is due to depressor effects from the vagus as peristalsis is not quickened after double vagotomy (30). More probably the longer intervals between beats are needed for adequate rest and recovery, so that the muscle can maintain a constant level of efficiency. For the same reason, the medusae pulsate normally at only about one-seventh the rate that they are capable of maintaining under certain conditions (31).

ANATOMICAL DIFFERENCES

It is hoped that this work may induce histologists to seek for regional differences in the nervous and muscular tissues of the stomach, and to study more closely the region about the cardia and the lesser curvature. Openchowski years ago claimed that the automatism of the cardia is due to groups of peculiar ganglion cells under the serosa (32). These cells were like those found in the heart. They were distinct from Auerbach's plexus; and when they were stripped off, automatic movements ceased. This, of course, might have been due to the trauma. He found similar groups of cells near the pylorus, but there were very few in the body of the stomach. Schütz also has described such ganglia grouped about the cardia and pylorus (33). Those in the pyloric portion and fundus had connective tissue capsules. Near the cardia the cells were not in the muscle layers, as they were elsewhere in the stomach, but were in the connective tissue between the layers.

Keith (34), who has recently done some very interesting work on this problem, found the myenteric plexus well developed only in the pyloric division and along the lesser curvature. There was no localized increase or development at the point where gastric movements ordinarily seem to begin. There was, however, a "distinct modification of the musculature and myenteric plexus just distal to the ring which

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marks the cessation of the esophageal epithelium and the commencement of the gastric lining. At that site there was a definite development of neuro-muscular junctional tissue—just such an area as might serve as a nodal center for the stomach." In this region in the echidna he found tissue similar to that seen in the sino-auricular node of the same animal. He believes the contractions of the stomach are there initiated. Thus, reasoning along the same lines but using different methods, Doctor Keith and I have arrived, independently, at the same conclusion.

Other differences will probably be found in the muscle itself. It is well known that there are marked differences in irritability, latent period and form of the contraction-curve between the pale and red voluntary muscles in the same frog or rabbit, between the flexors and extensors, between the abductor and adductor of the crab's pincers, or between the wing and leg muscles of an insect. Histological differences have also been found corresponding to the functional ones. The proportion between the sarcoplasm and the fibrils varies markedly in different muscles; and even in the same muscle there may be fine and coarse fibers with different degrees of irritability, so that weak and strong stimuli produce different effects. After reading the articles of Ranvier (35), Rollett (36), Grützner (37), and particularly that of Paukul (38), and seeing how remarkably striated muscles vary, not only throughout the animal kingdom but in the individual body, it seems to me unreasonable to expect that smooth muscle should have fixed properties and structure. Marked differences in the physiological properties of bits of smooth muscle from different organs are well known, but I can find very little about histological differences. McGill is about the only one who seems to have observed such details. She found in some parts of the digestive tract a persistence of the embryonic condition as shown by the distinct syncytial arrangement of the muscle fibers with both end and side anastomoses (39). Unfortunately, Doctor McGill had no reason then to note just where those bodies of embryonic tissue were found.

SUMMARY

The evidence presented suggests that the gastro-intestinal tube may originally have been constructed so that the rhythmicity of any one segment varied inversely as the distance from the pharynx.

It is proposed to study the stomach from the point of view that it has been evolved from a primitive tube much as the heart has been en-

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larged and specialized. Reasoning from the grounds of comparative anatomy and embryology, we should expect to find the remnants of this tube along the lesser curvature of the stomach from the cardia to the pyloric antrum.

Excised strips of muscle from the cardiac end, and particularly that one on the lesser curvature next to the cardia, show the strongest tendency to rhythmic contraction.

Different types of tracings are peculiar to the strips from different regions of the stomach. Speaking roughly, the rate of contraction varies inversely as the distance from the cardia. The tone seems to be higher on the lesser than on the greater curvature.

Strips from the human stomach behave very similarly to those obtained from the rabbit, cat and dog.

The differences observed in the strips probably determine the direction and local peculiarities of the peristaltic wave as it sweeps over the stomach.

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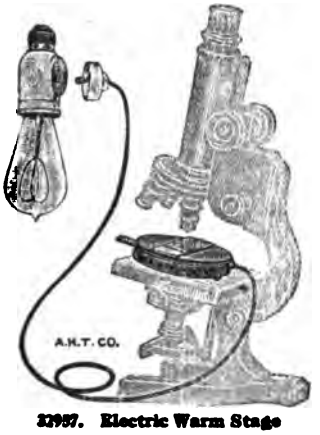
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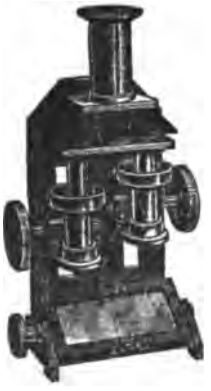
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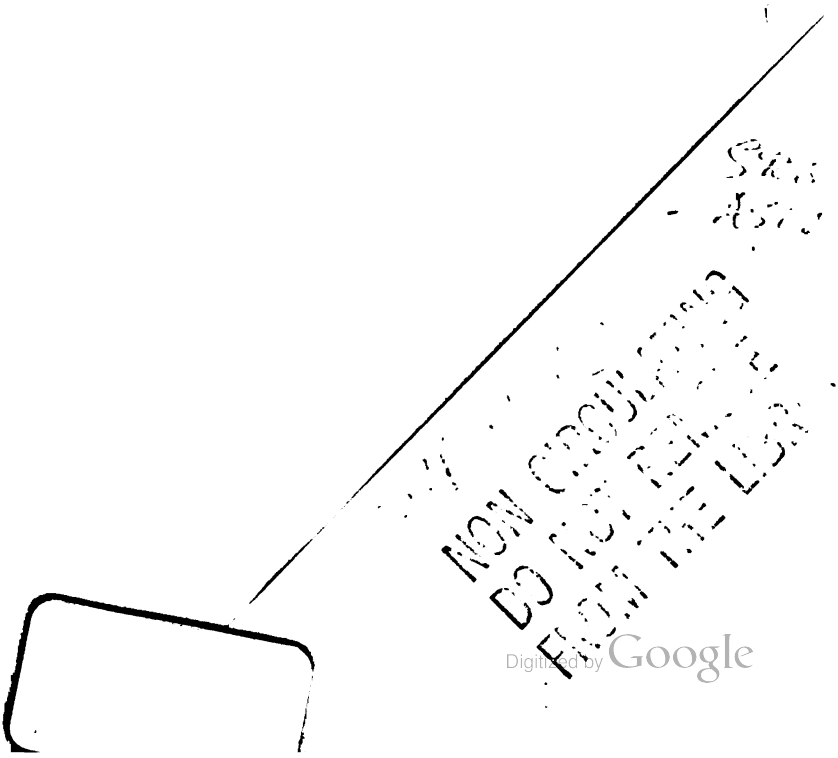
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